

# BEST AVAILABLE COPY

WO 02/46472

PCT/US01/46418

phosphate buffer.

621. The method of claim 619 wherein the salt is sodium chloride in a phosphate buffer.

5

622. The method of Claims 599 or 607 wherein the nanoparticles have a diameter ranging between about 10 and about 100 nm.

623. The method of Claims 599 or 607 wherein the nanoparticles have a diameter of about 50 nm.

10

624. The method of Claims 599 or 607 wherein the nanoparticles have a diameter of about 100 nm.

625. The method of Claims 599 or 607 wherein two scattered light detectable nanoparticle probes of different diameters are used.

15

626. The method of claim 624 wherein the nanoparticle probes have a diameter of 50 nm and 100 nm.

20

25

FIG. 1

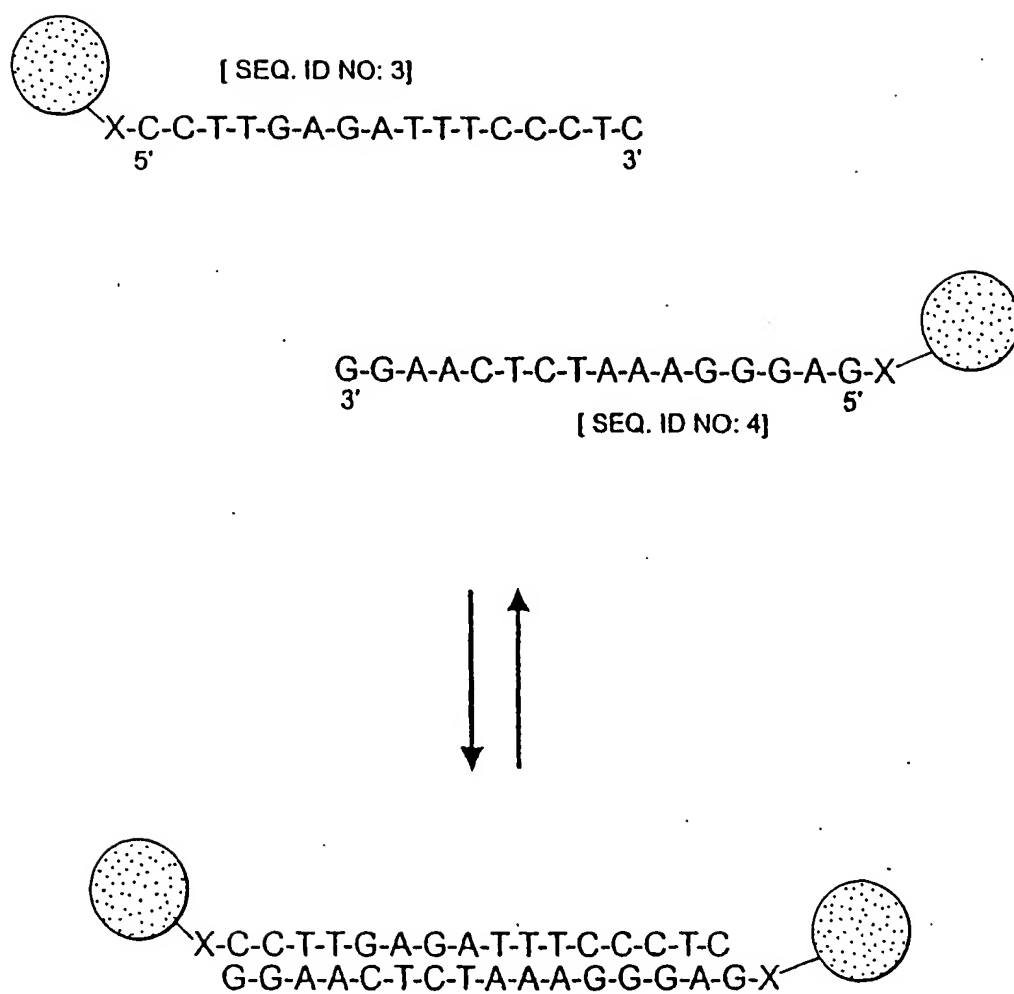


FIG. 2

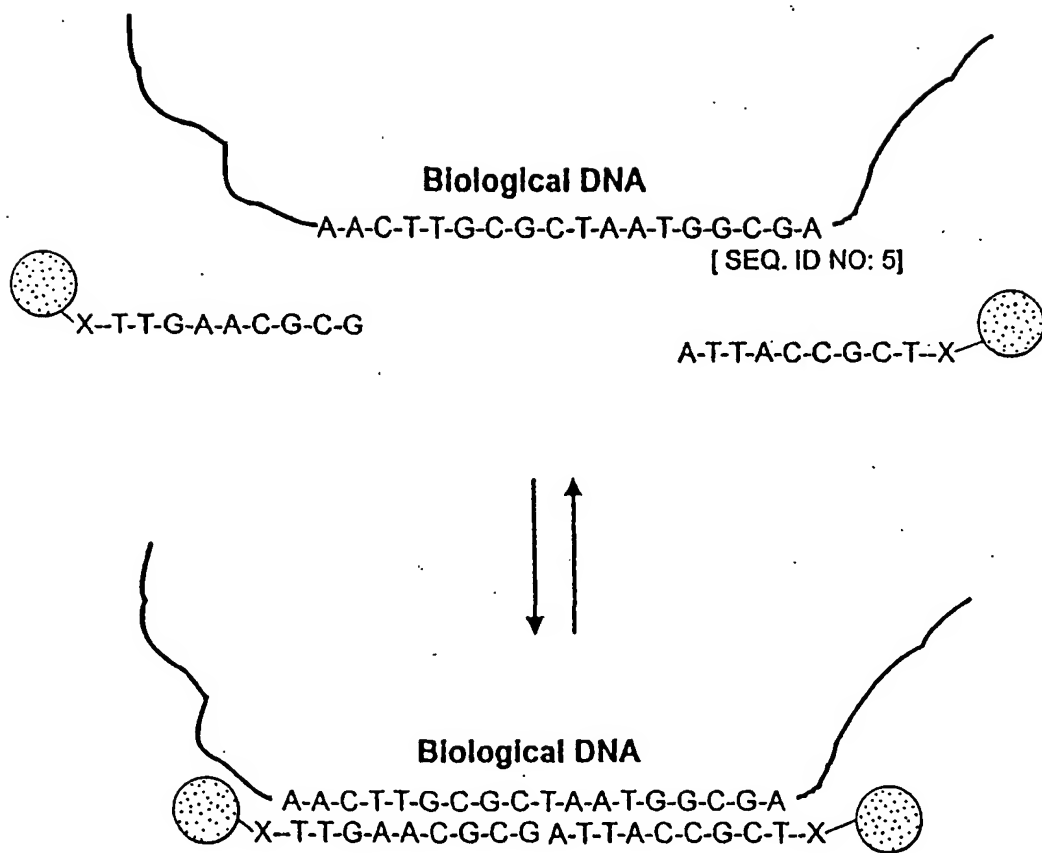
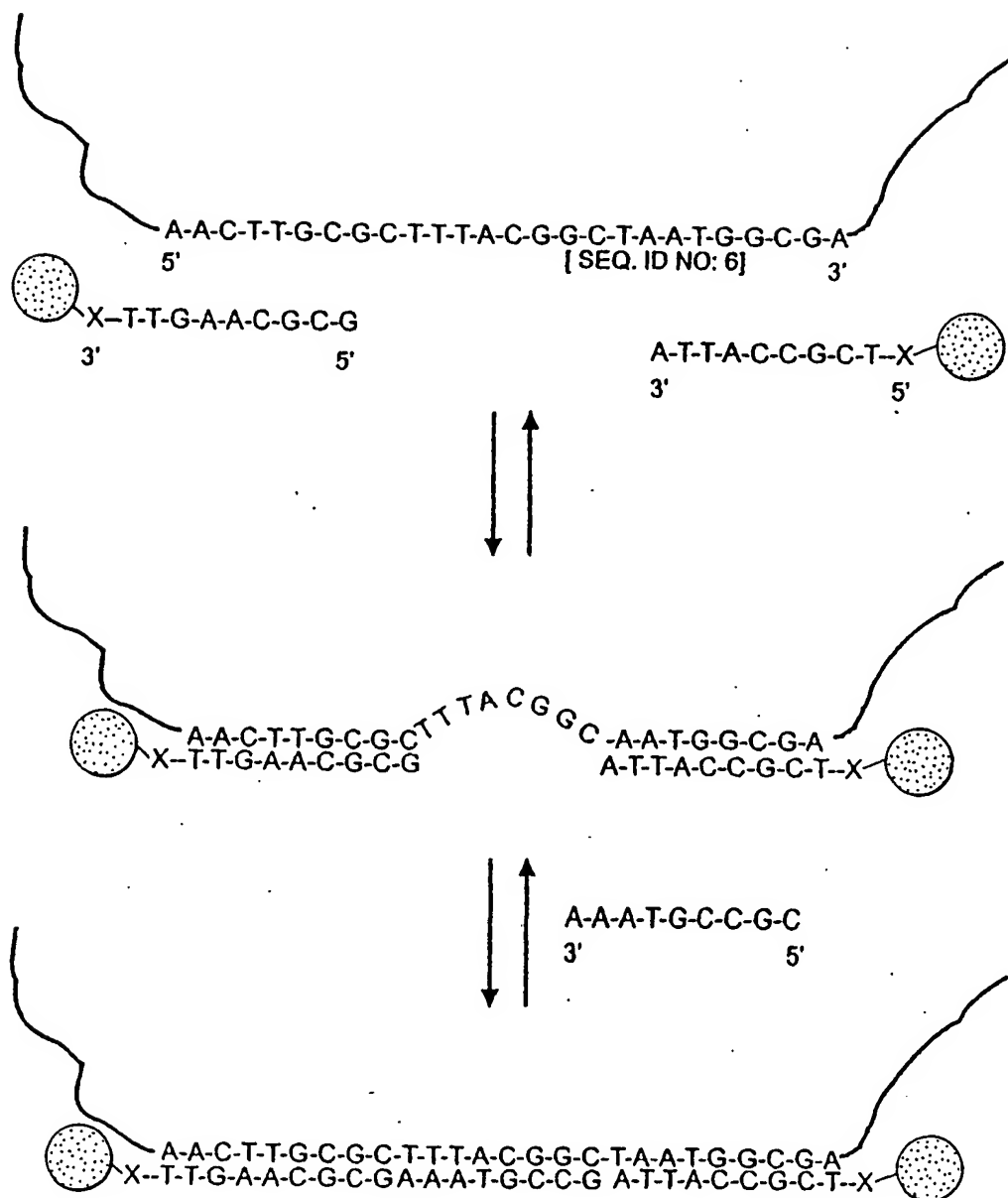


FIG. 3



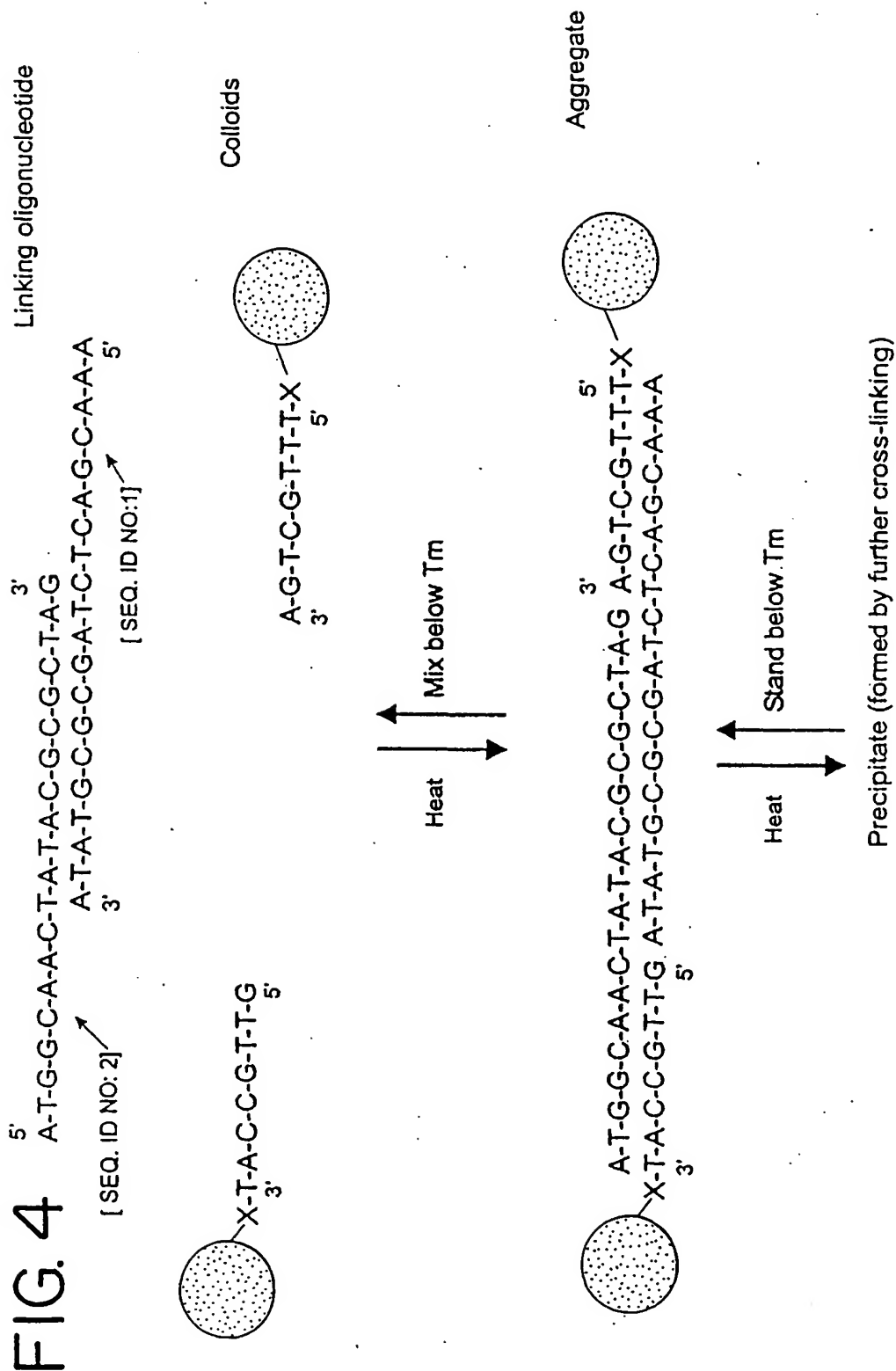


FIG. 5

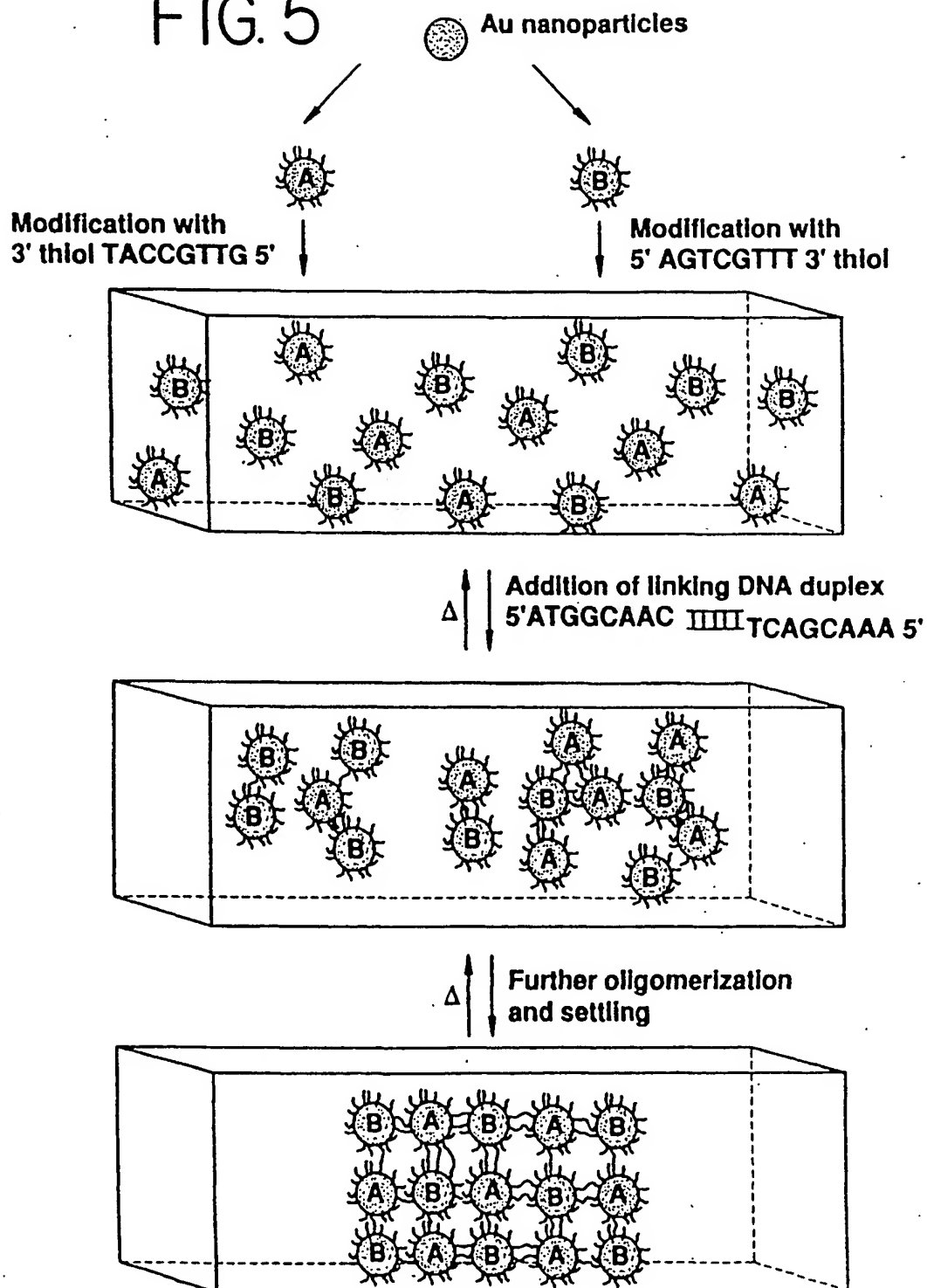


FIG. 6A FIG. 6B FIG. 6C

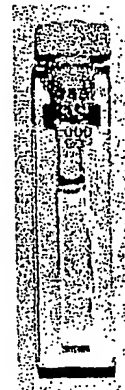
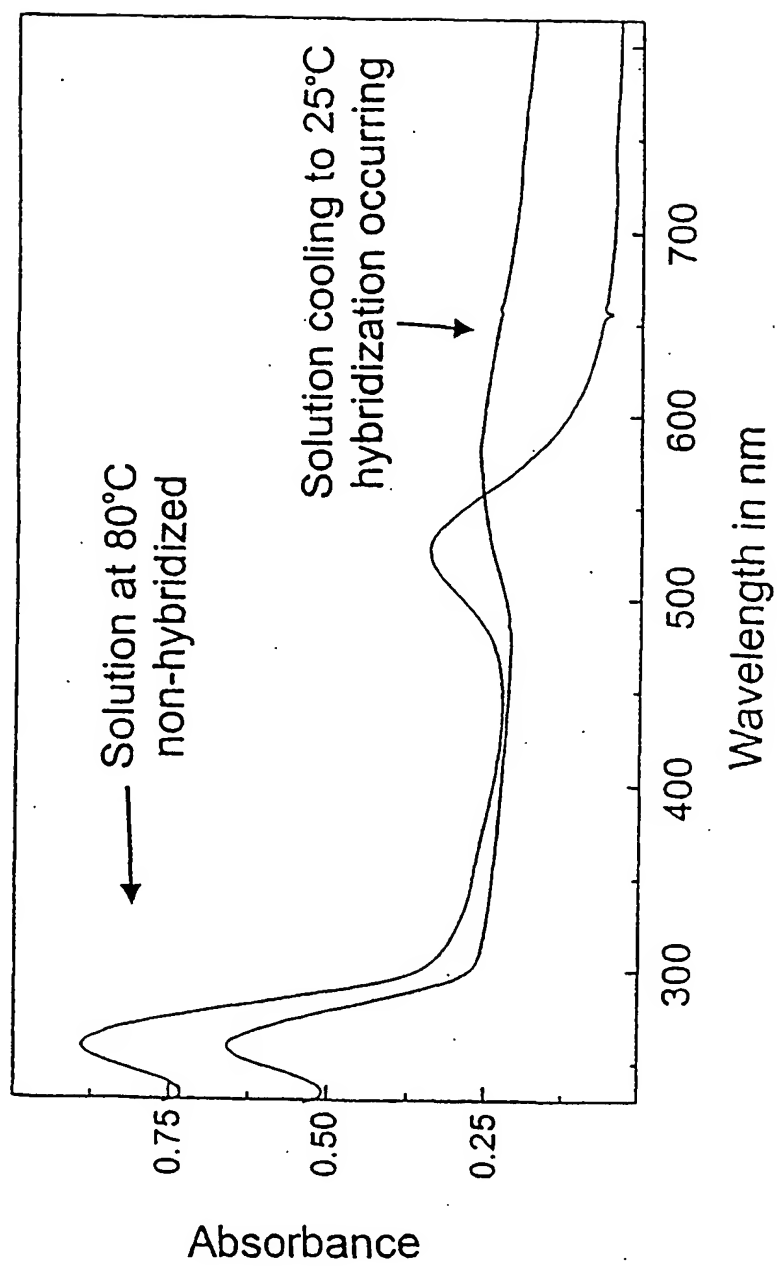


FIG. 7





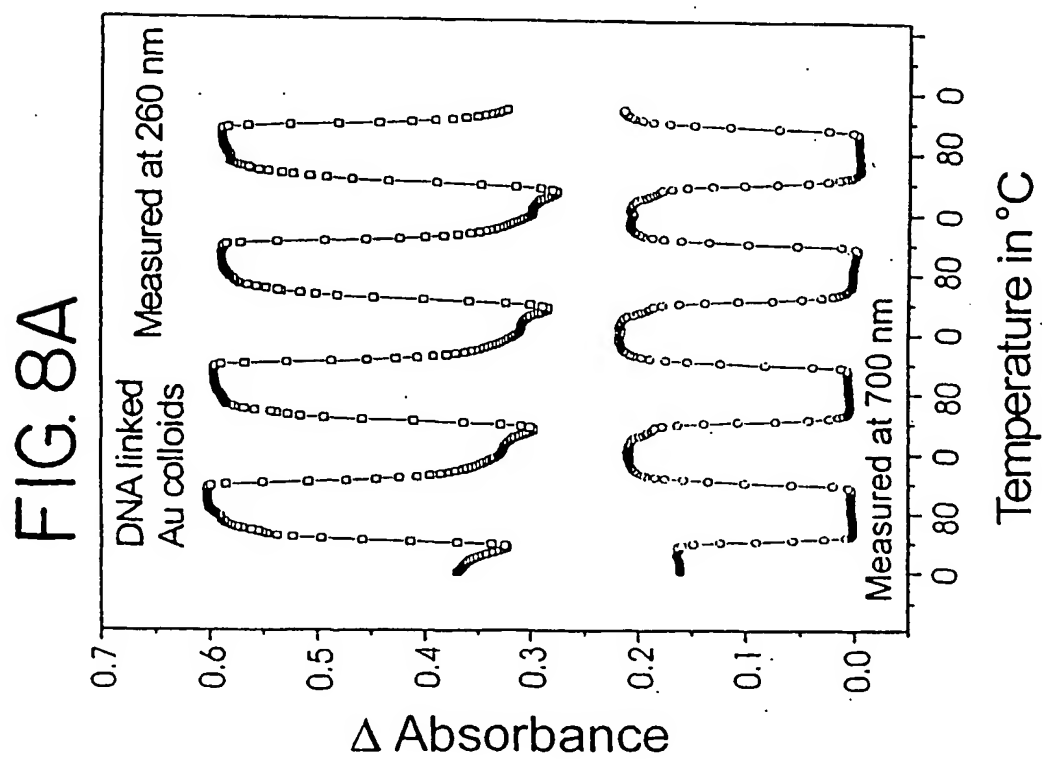
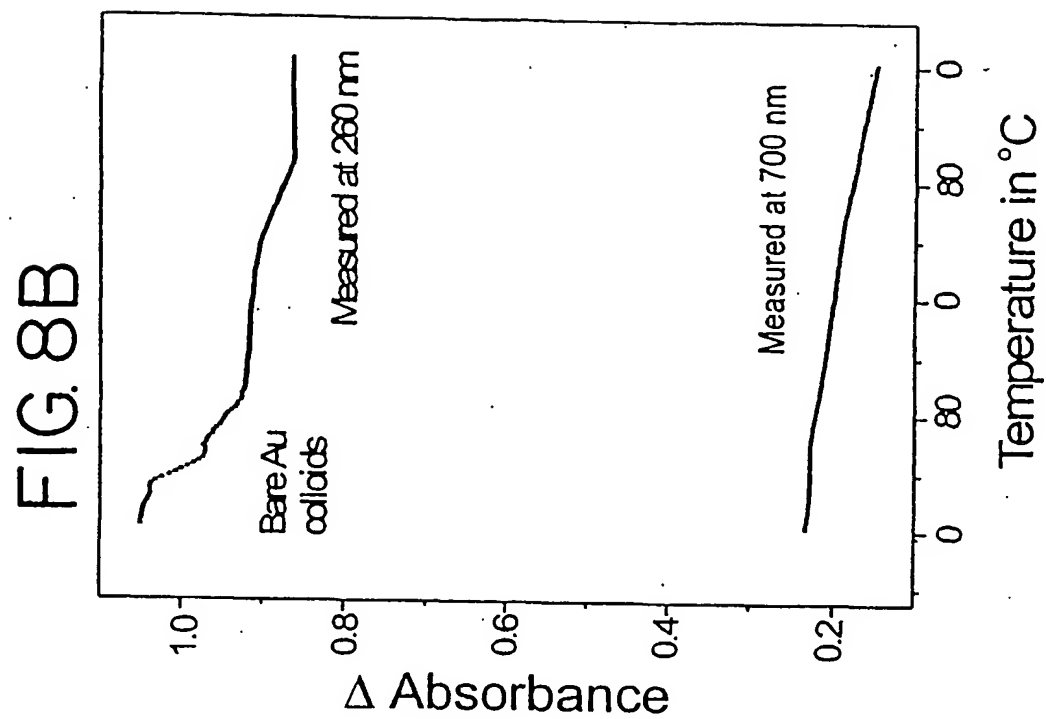


FIG. 9A

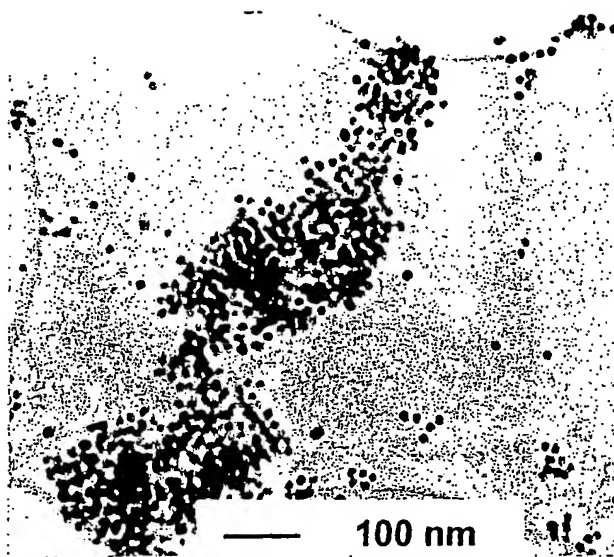


FIG. 9B

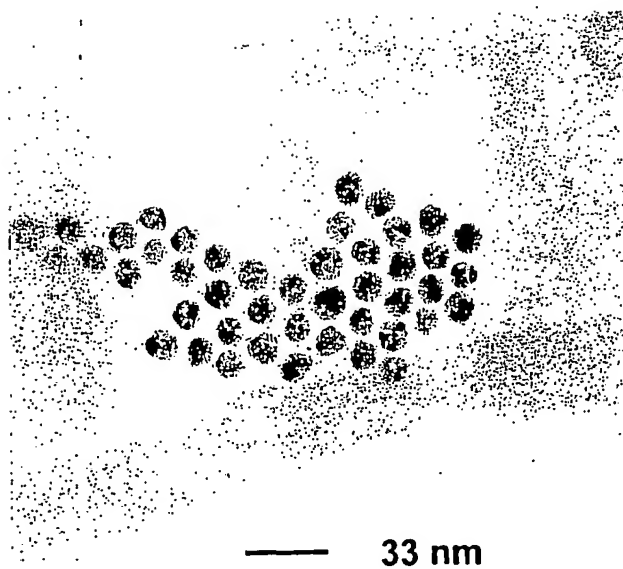


FIG. 10

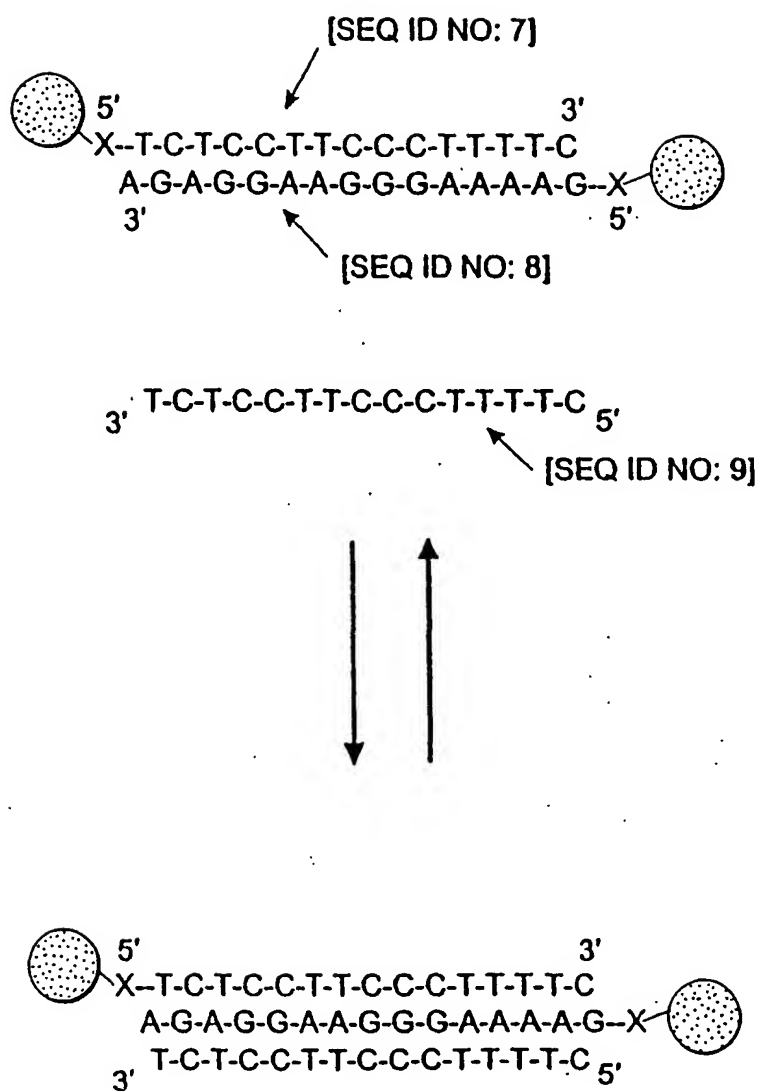
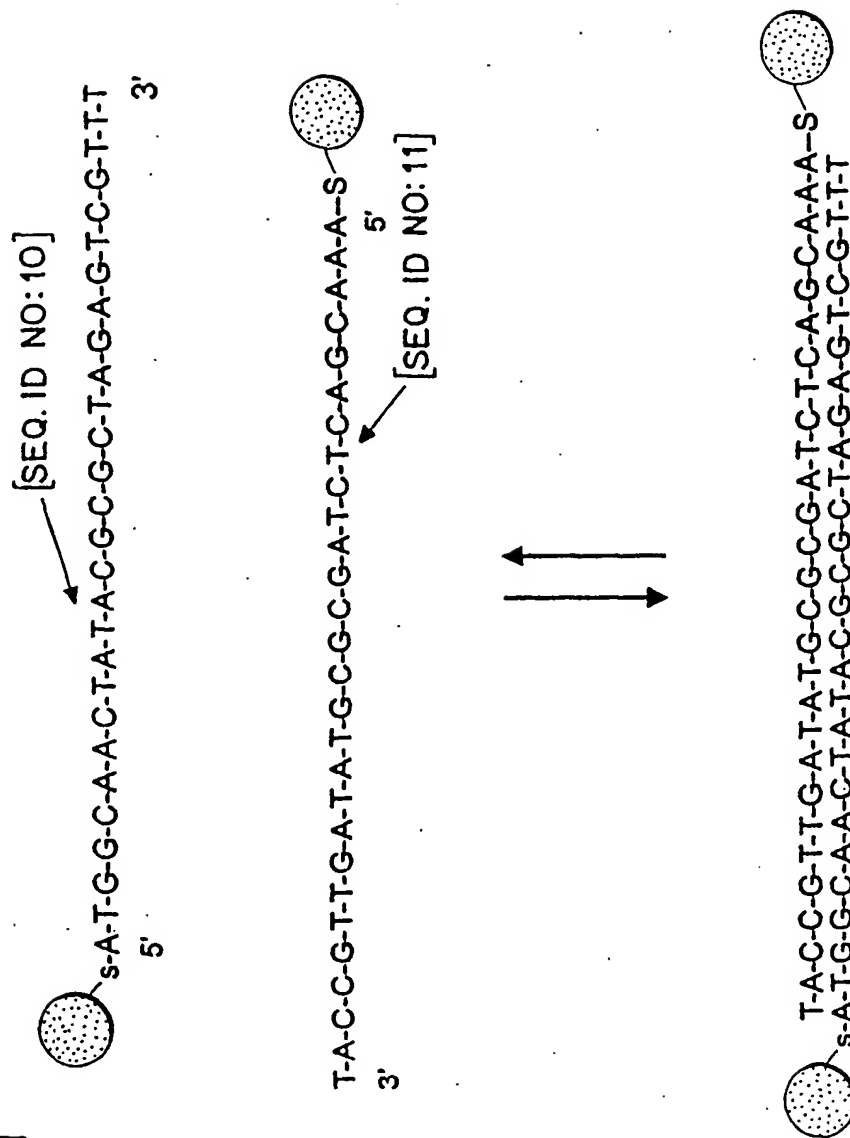
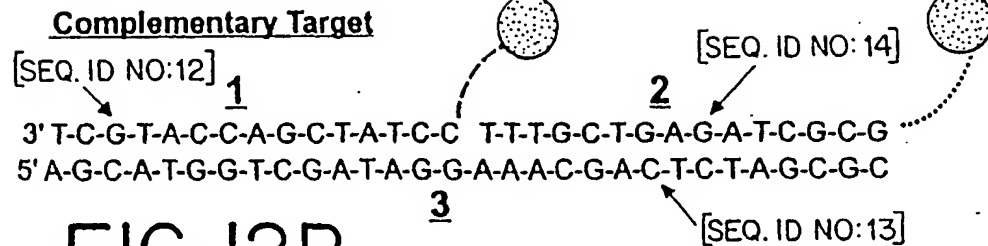


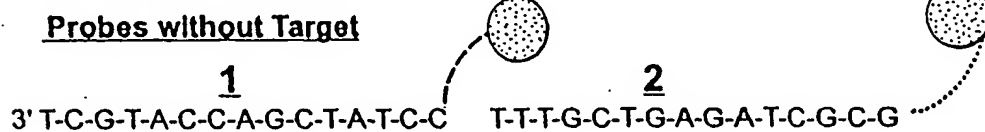
FIG. 11



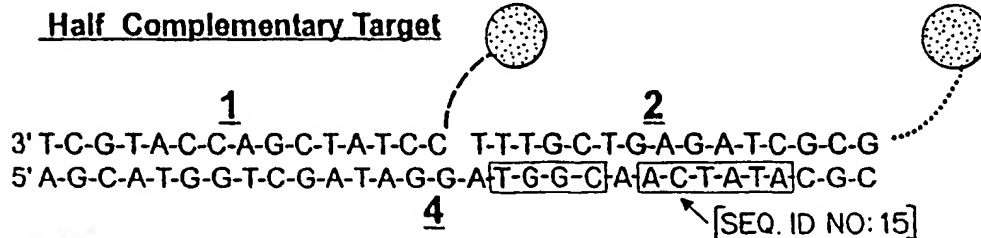
# FIG. 12A



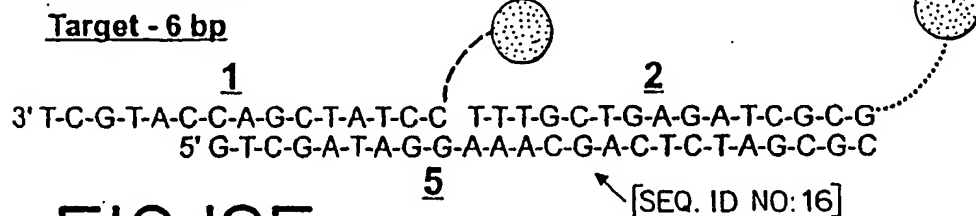
# FIG. 12B



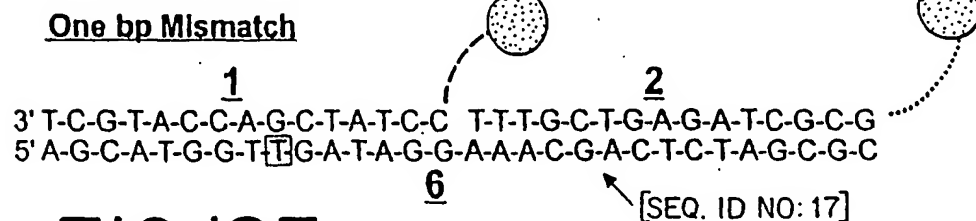
# FIG. 12C



# FIG. 12D



# FIG. 12E



# FIG. 12F

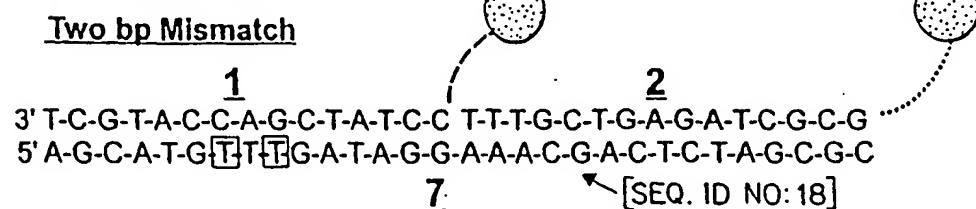


FIG. 13A

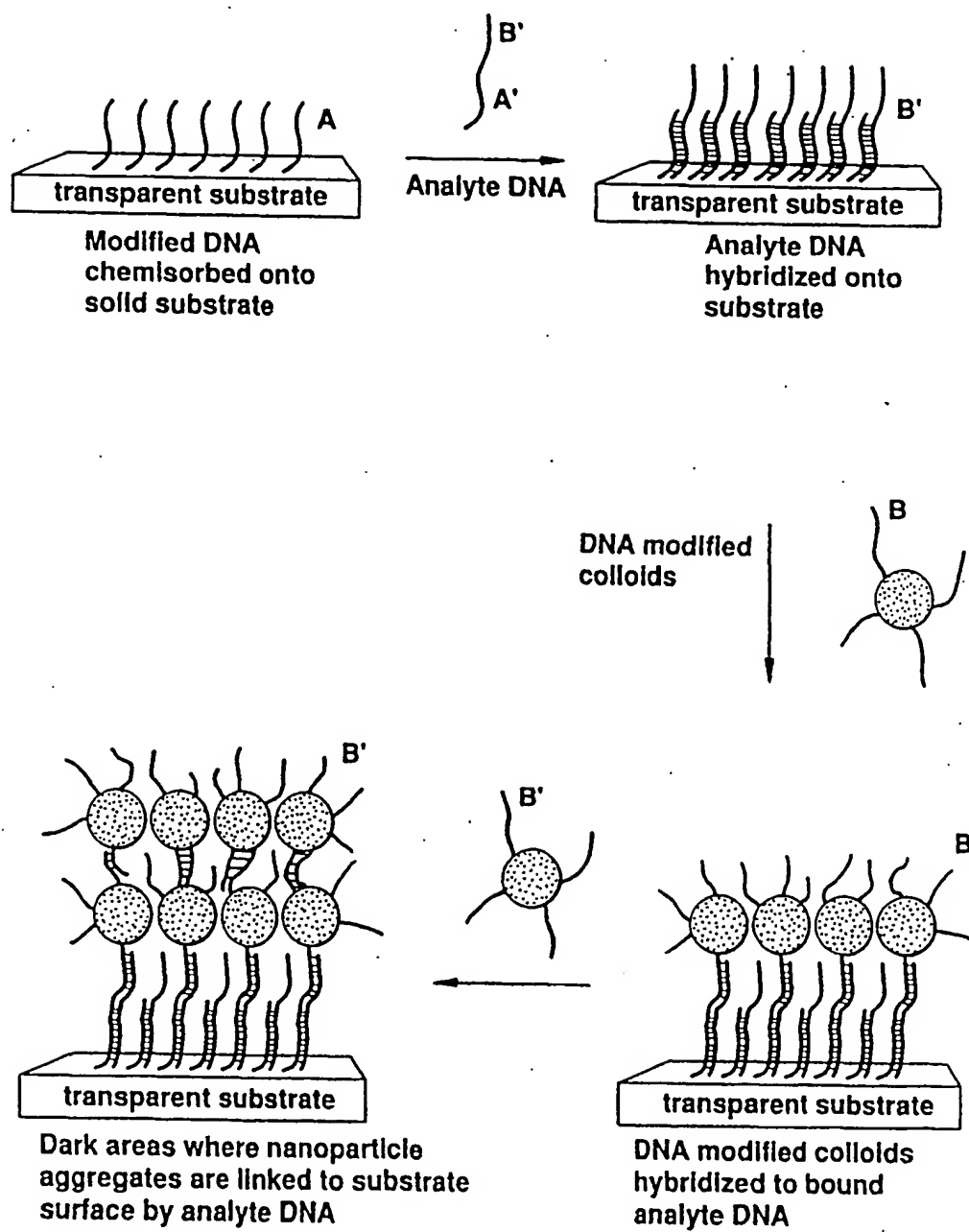


FIG. 13B

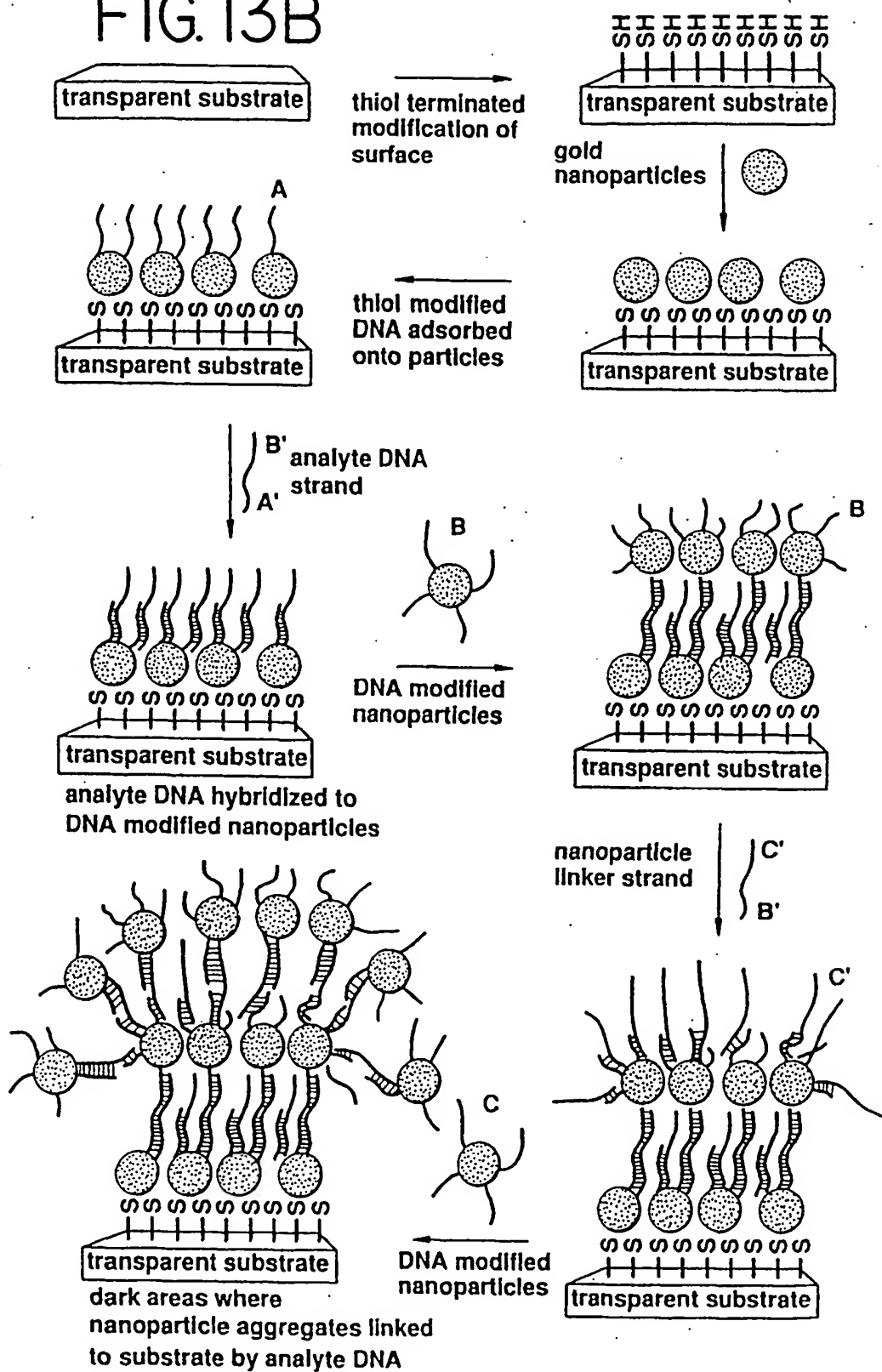


FIG. 14A

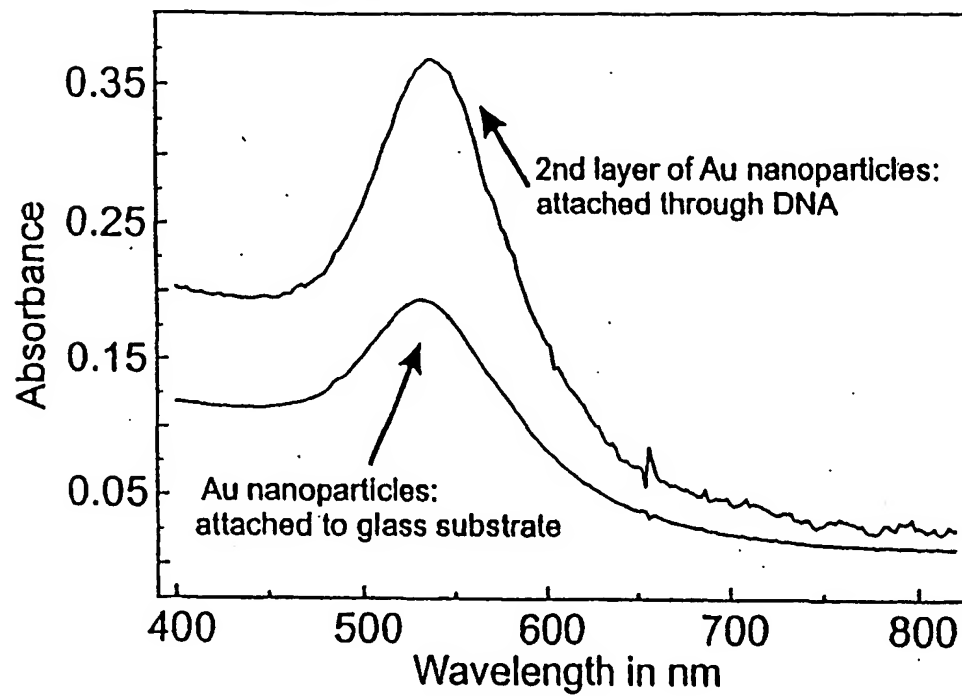


FIG. 14B

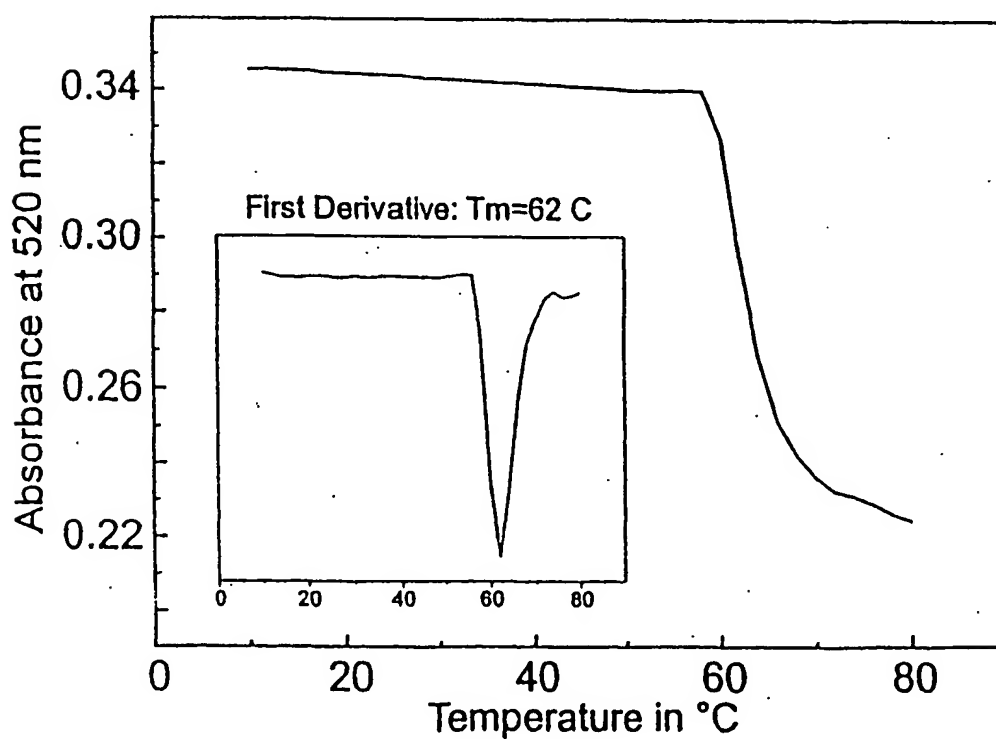




FIG. 15A

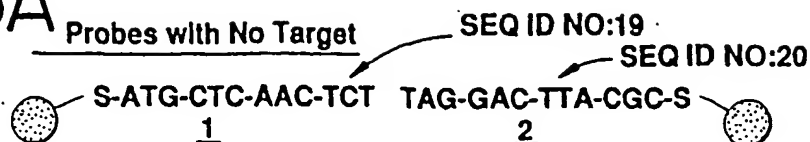


FIG. 15B

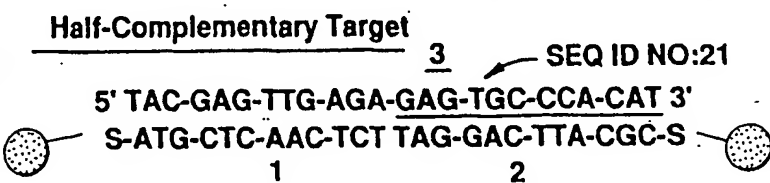


FIG. 15C

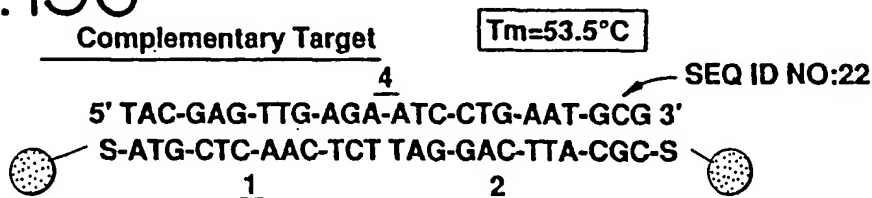


FIG. 15D

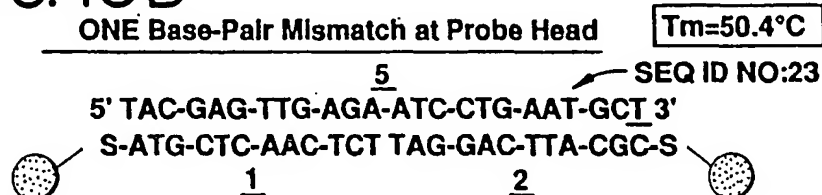


FIG. 15E

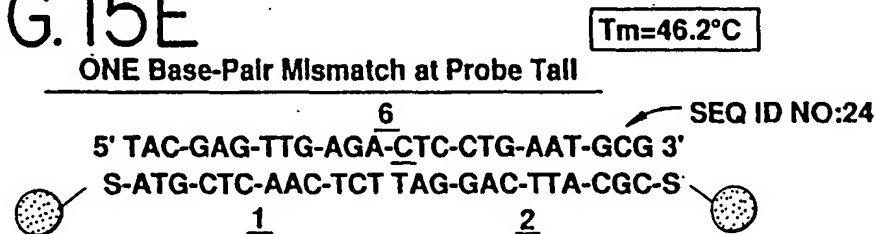


FIG. 15F

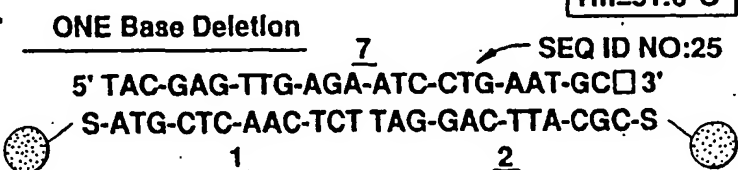
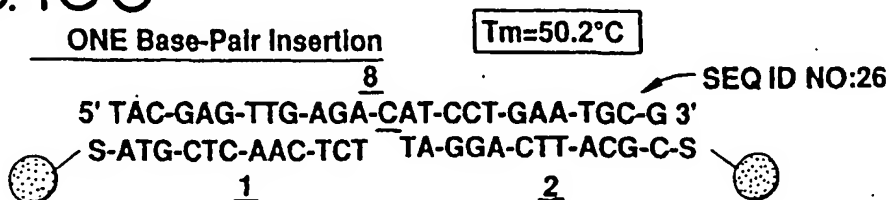


FIG. 15G



## FIG. 16A

24 Base Template

5' TAC-GAG-TTG-AGA-ATC-CTG-AAT-GCG 3'  
 —S-ATG-CTC-AAC-TCT TAG-GAC-TTA-CGC-S —  
 1 2

## FIG. 16B

48 Base Template with Complementary 24 Base Filler

5' TAC-GAG-TTG-AGA-CCG-TTA-AGA-CGA-GGC-AAT-CAT-GCA-ATC-CTG-AAT-GCG 3'  
 —S-ATG-CTC-AAC-TCT GGC-AAT-TCT-GCT-CCG-TTA-GTA-CGT TAG-GAC-TTA-CGC-S —  
 1 2

## FIG. 16C

72 Base Template with Complementary 48 Base Filler

5' TAC-GAG-TTG-AGA-CCG-TTA-AGA-CGA-GGC-AAT-CAT-GCA-TAT-ATT-GGA-CGC-TTT-ACG-GAC-AAC-ATC-CTG-AAT-GCG 3'  
 —S-ATG-CTC-AAC-TCT GGC-AAT-TCT-GCT-CCG-TTA-GTA-CGT-ATA-TAA-CCT-GCG-AAA-TGC-CTG-TTG TAG-GAC-TTA-CGC-S —  
 1 2

FIG. 17B

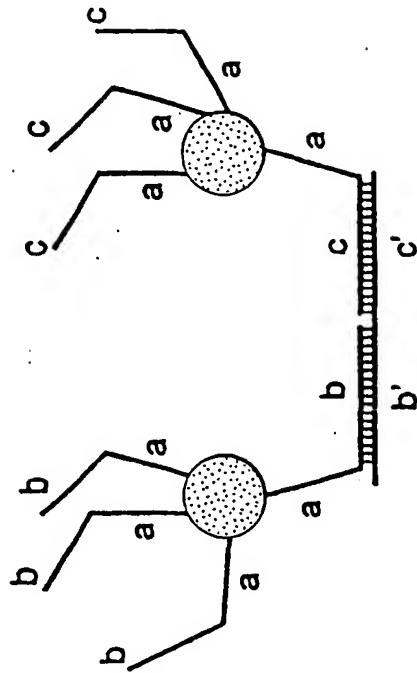


FIG. 17A

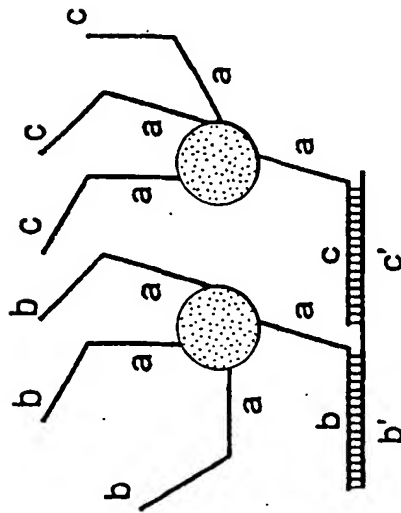


FIG. 17C

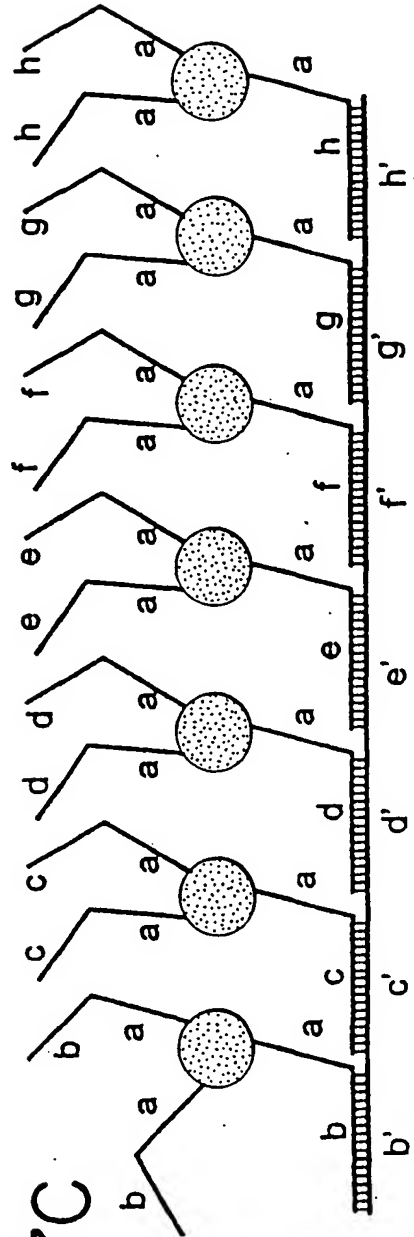


FIG. 17D

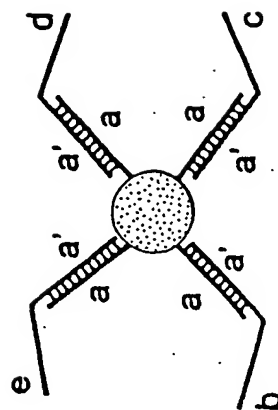
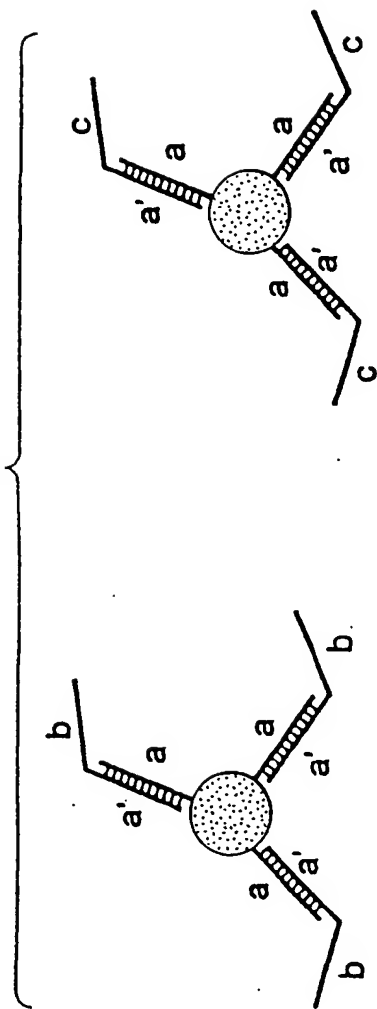


FIG. 17E

FIG. 18

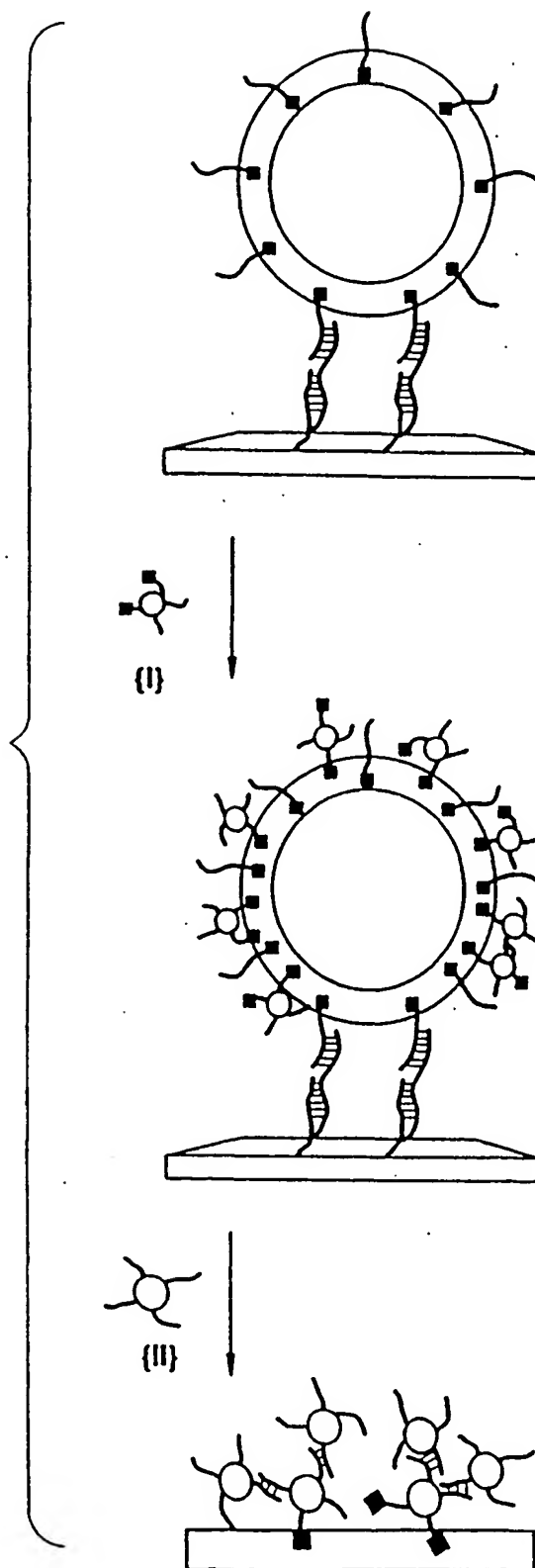


FIG. 19A

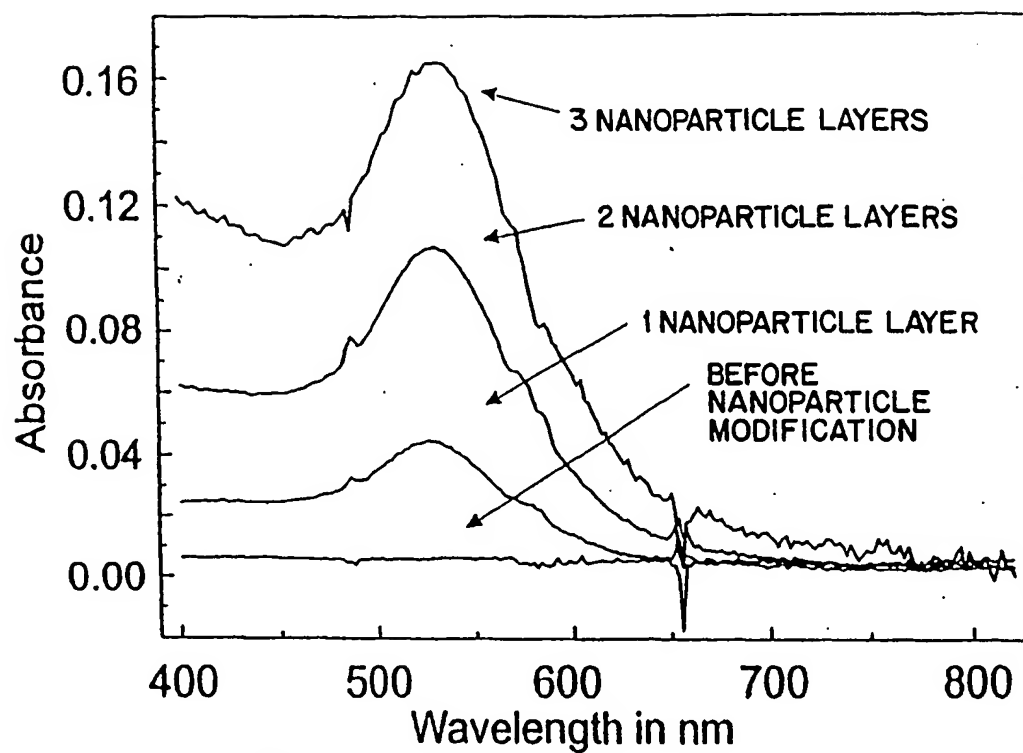


FIG. 19B

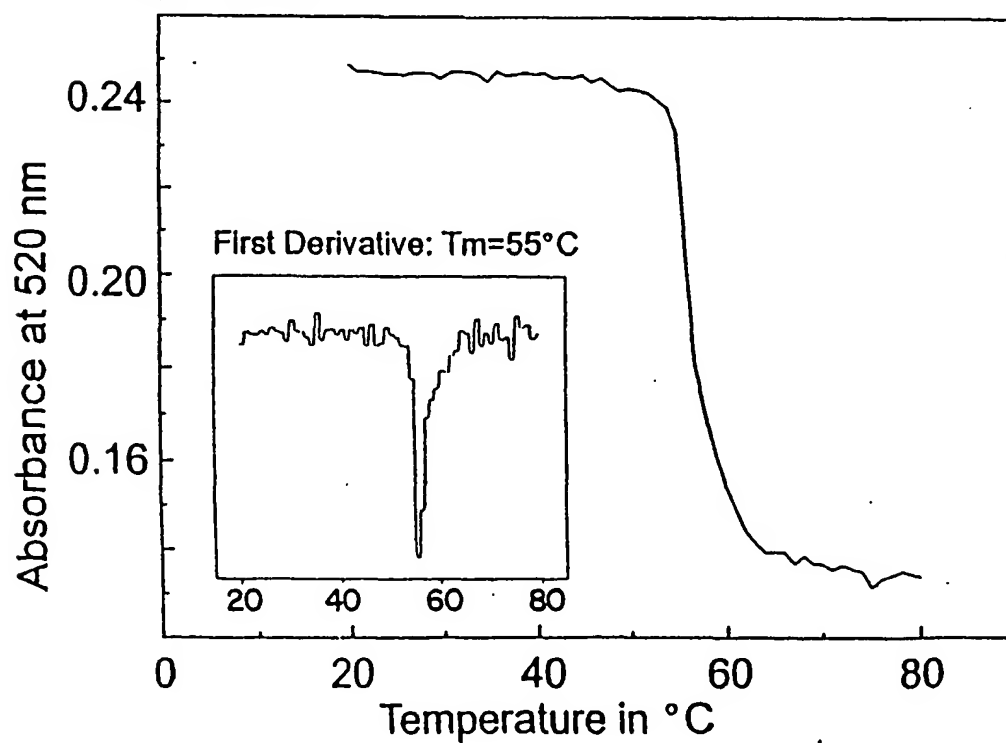


FIG. 20A

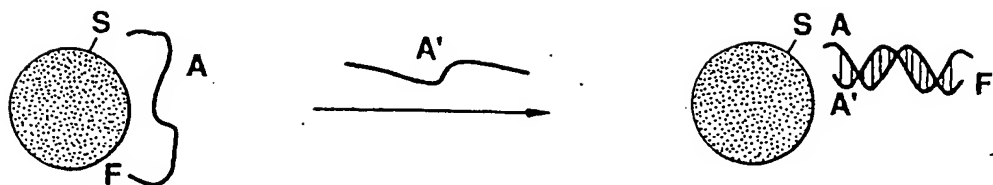


FIG. 20B

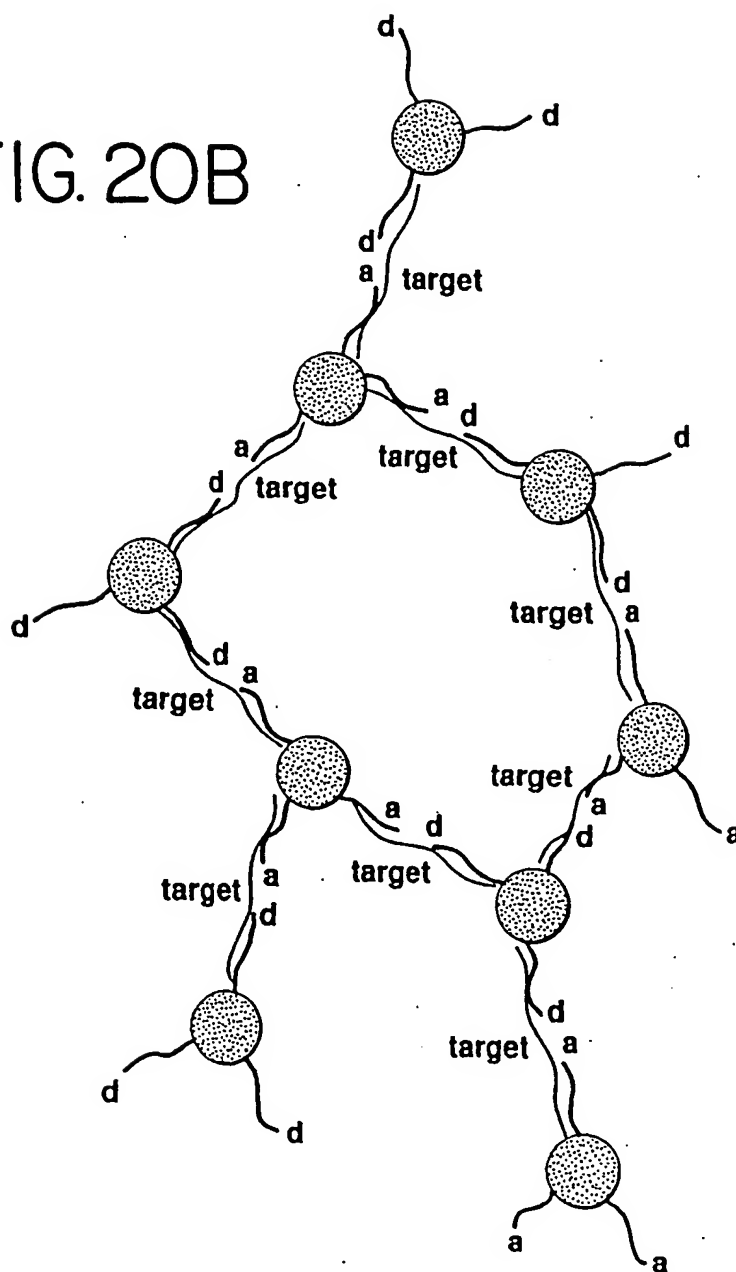


FIG. 21

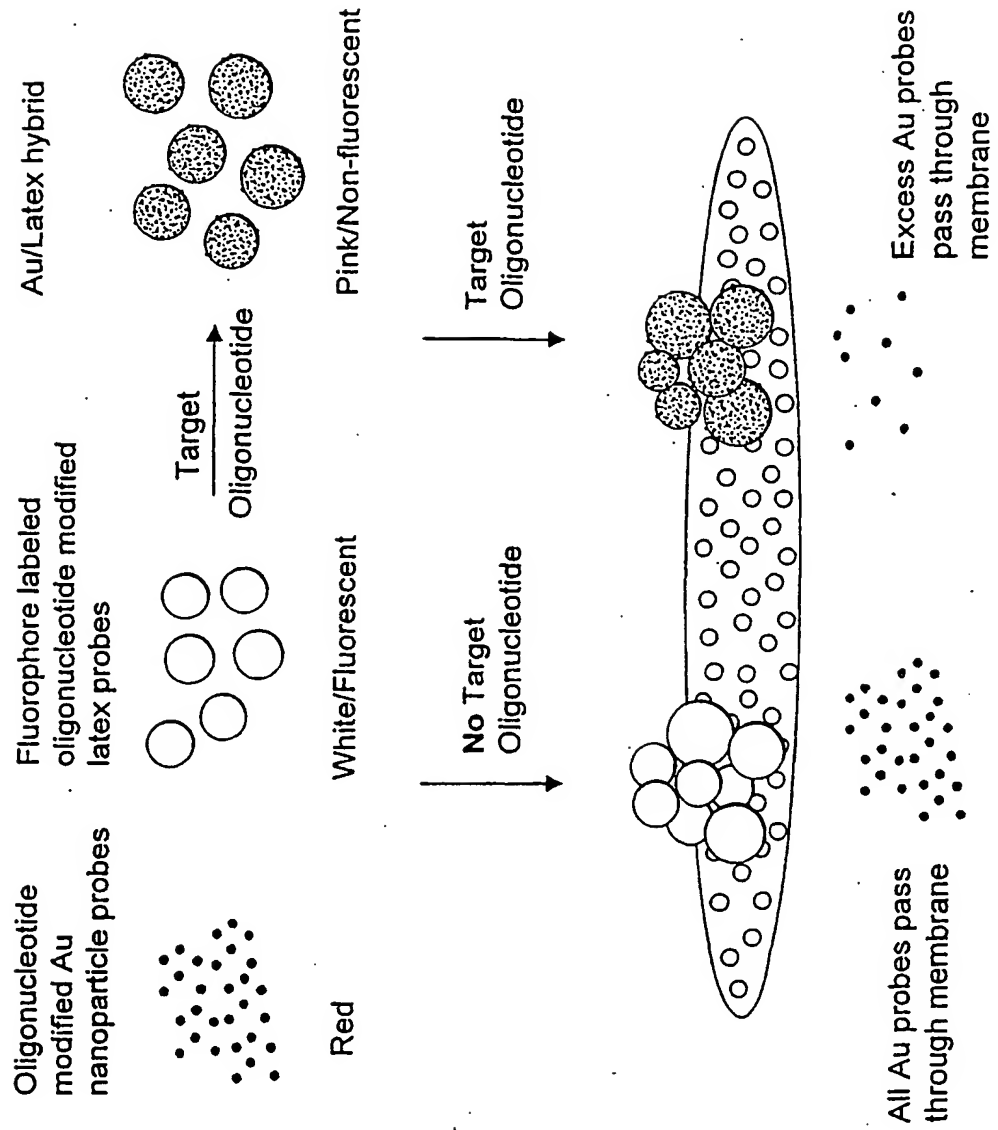
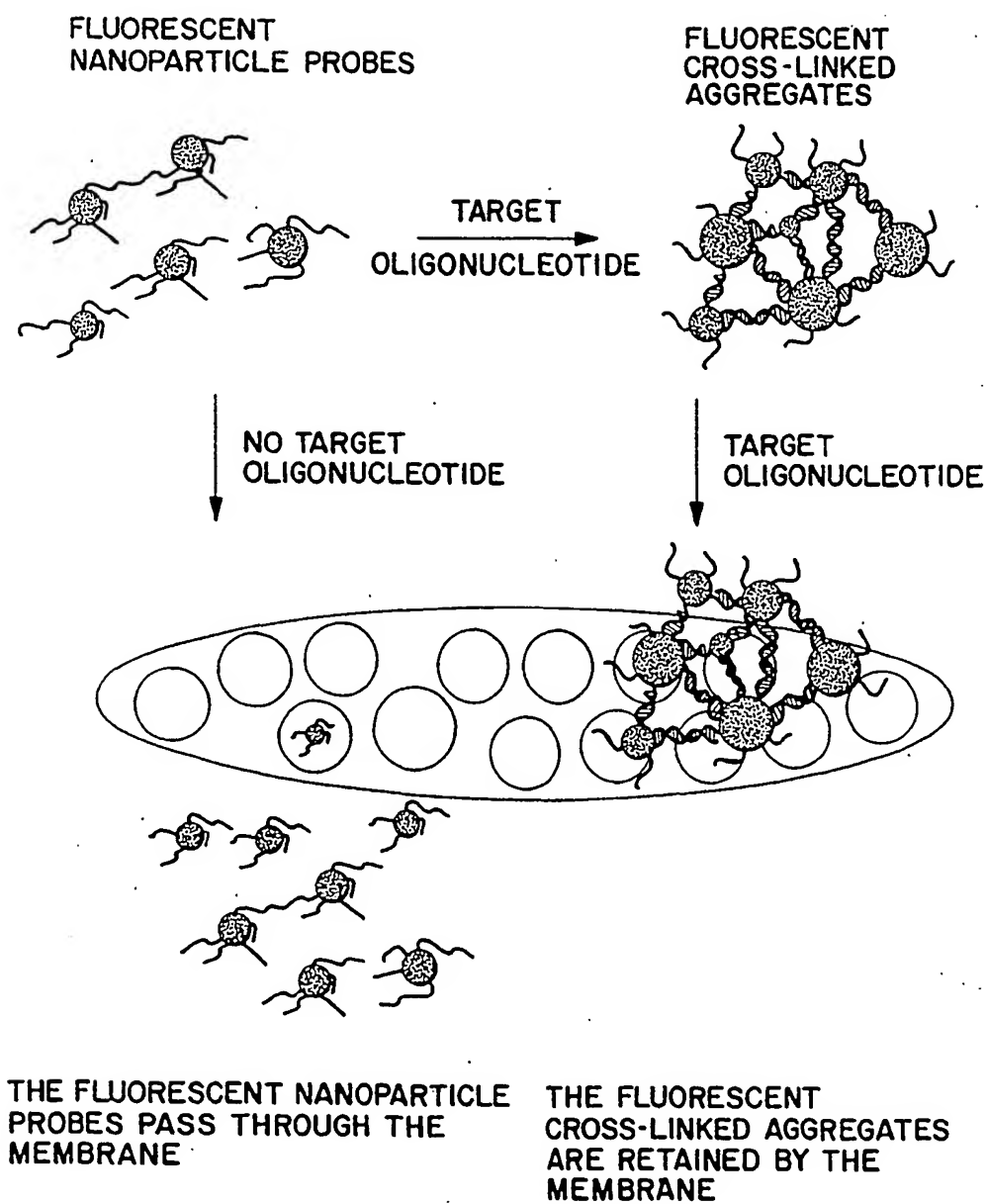




FIG. 22



# FIG. 23

## Anthrax PCR Product

5'G GCG GAT GAG TCA GTA GTT AAG GAG GCT CAT AGA GAA GTA ATT AAT  
3'C CGC CTA CTC AGT CAT CAA TTC CTC CGA GTA TCT CTT CAT TAA TTA

TCG TCA ACA GAG GGA TTA TTG TTA AAT ATT GAT AAG GAT ATA AGA AAA  
AGC AGT TGT CTC CCT AAT AAC AAT TTA TAA CTA TTC CTA TAT TCT TTT

ATA TTA TCC AGG GTT ATA TTG TAG AAA TTG AAG ATA CTG AAG GGC TT 3'  
TAT AAT AGG TCC CAA TAT AAC ATC TTT AAC TTC TAT GAC TTC CCG AA 5'

**141 mer Anthrax PCR product** [SEQ ID NO:36]

3' CTC CCT AAT AAC AAT — 

[SEQ ID NO:37]

3' TTA TAA CTA TTC CTA — 

[SEQ ID NO:38]

Oligonucleotide-Nanoparticle Probes

## Blocker Oligonucleotides

3' C CGC CTA CTC AGT CAT CAA TTC CTC CGA GT  
3' A TCT CTT CAT TAA TTA AGC AGT TGT  
3' TAT TCT TTT TAT AAT AGG TCC CAA TAT  
3' AAC ATC TTT AAC TTC TAT GAC TTC CCG AA

[SEQ ID NO:39]  
[SEQ ID NO:40]  
[SEQ ID NO:41]  
[SEQ ID NO:42]

FIG. 24

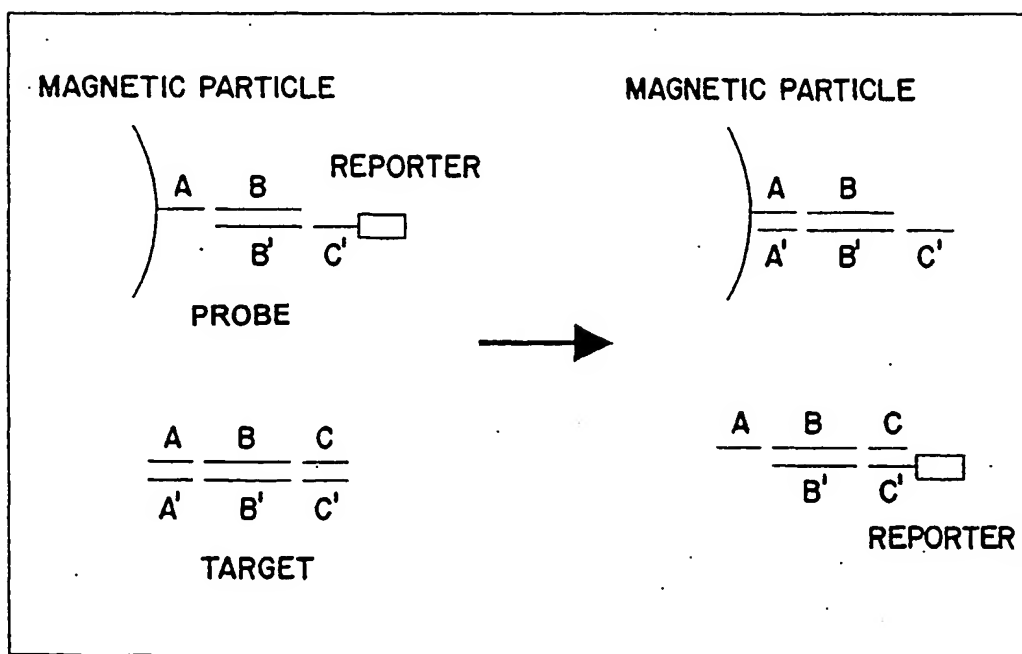
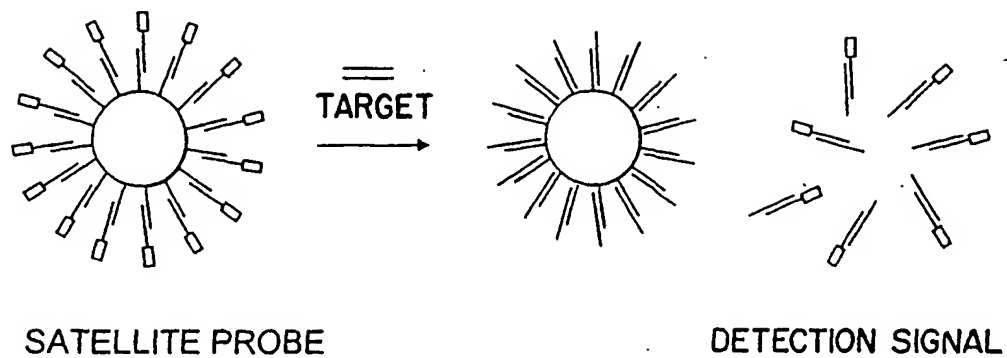


FIG. 25A

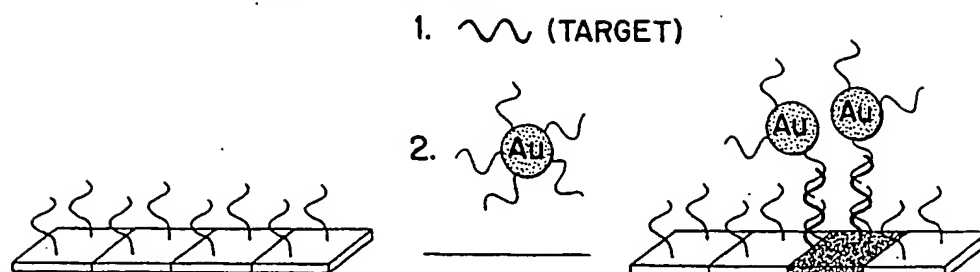


FIG. 25B

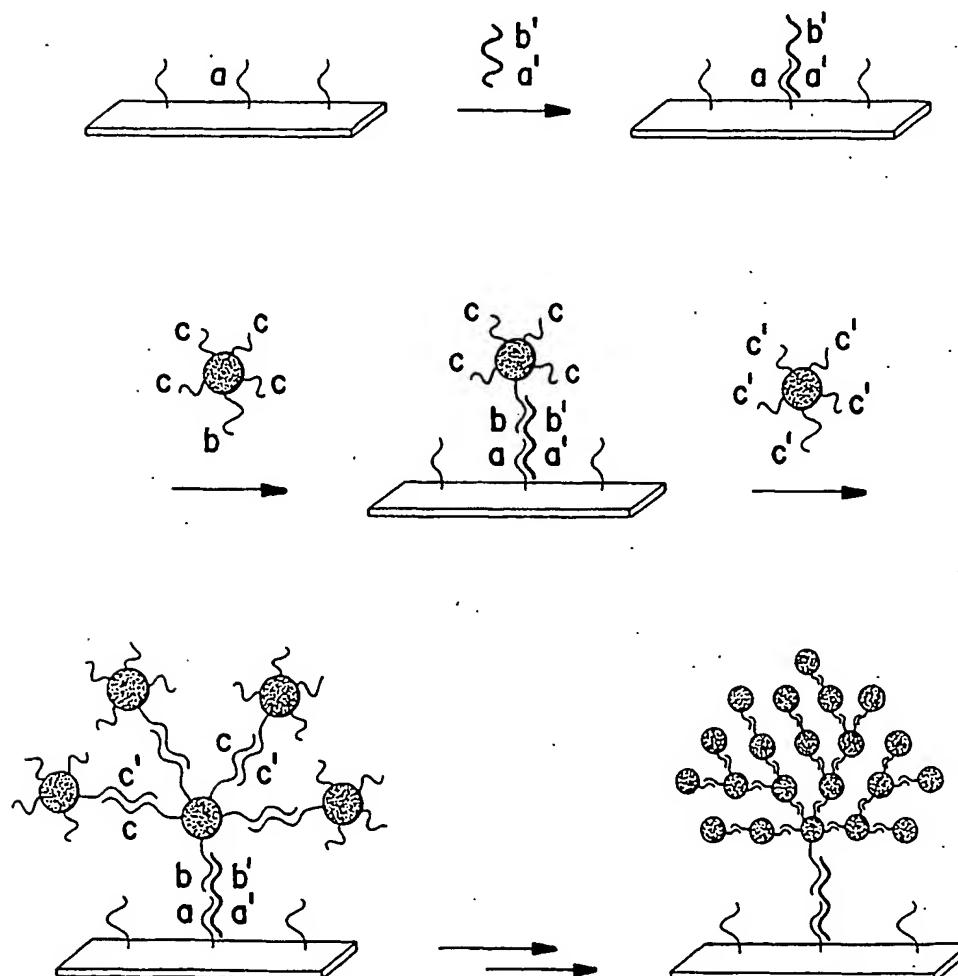


FIG. 26A

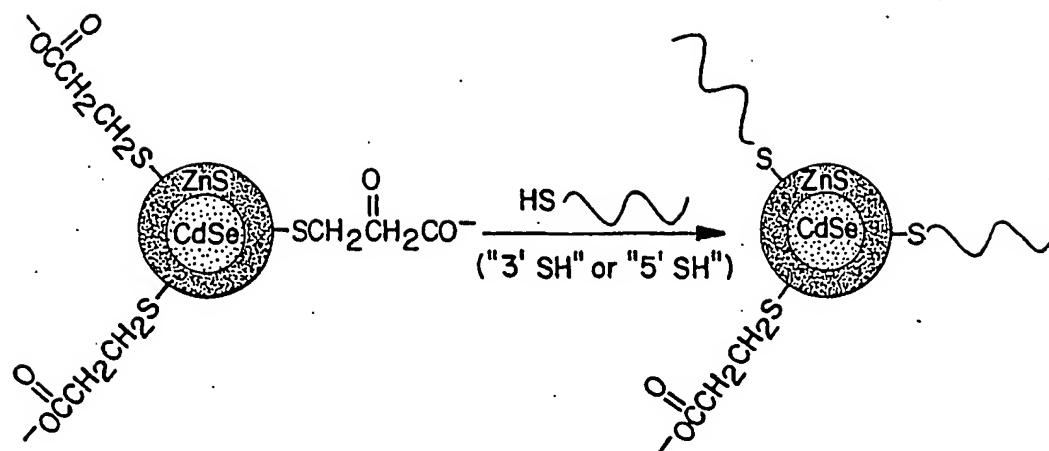


FIG. 26B

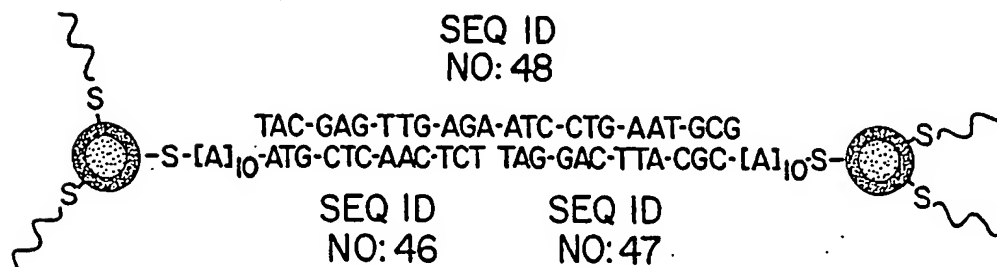


FIG. 27A

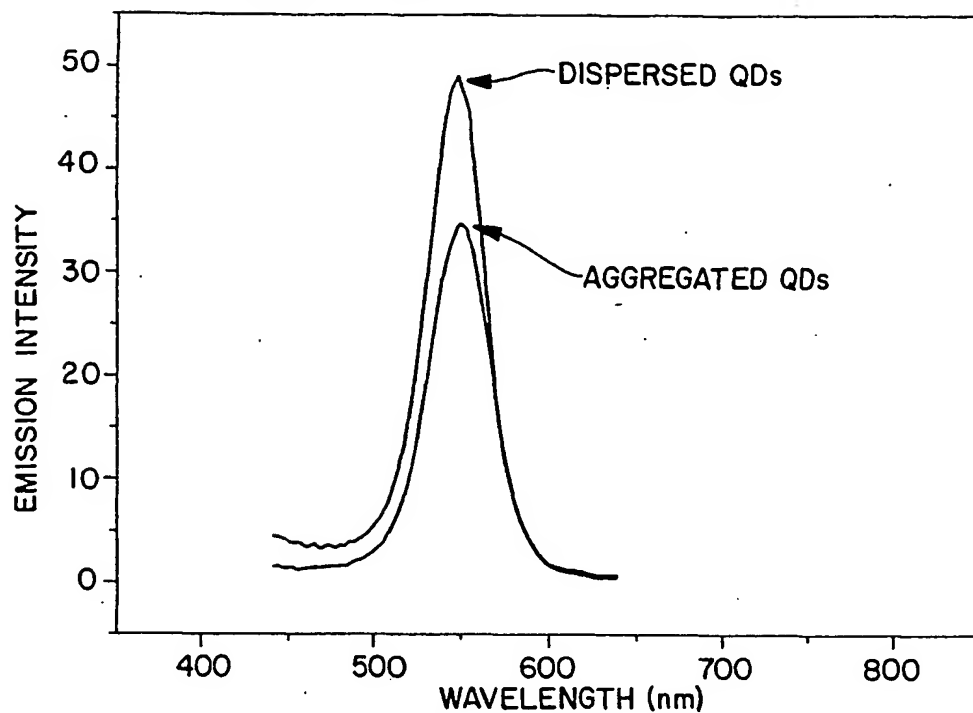


FIG. 27B

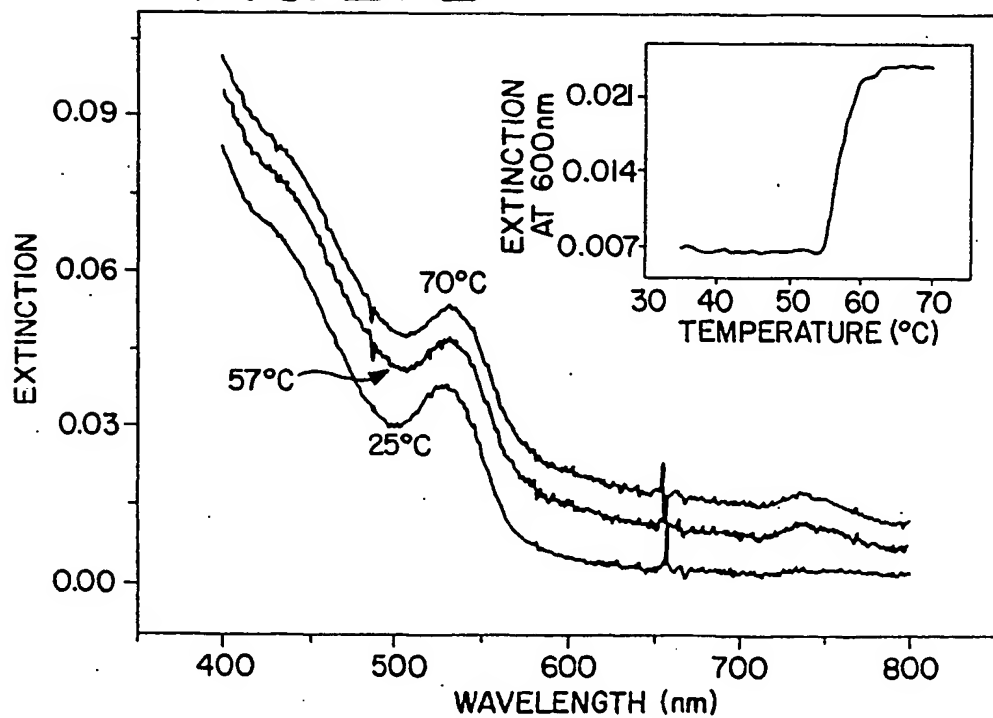


FIG. 27C

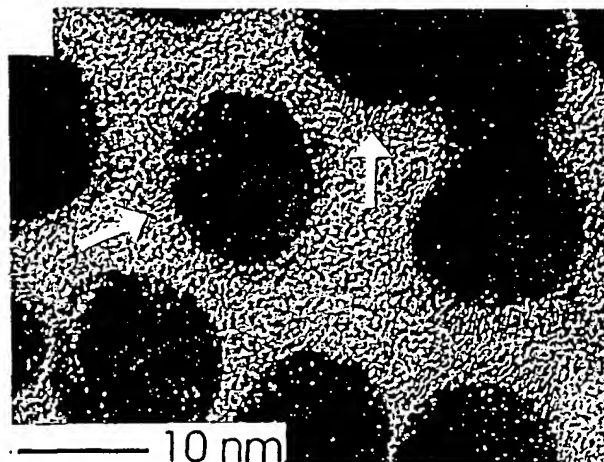


FIG. 27D

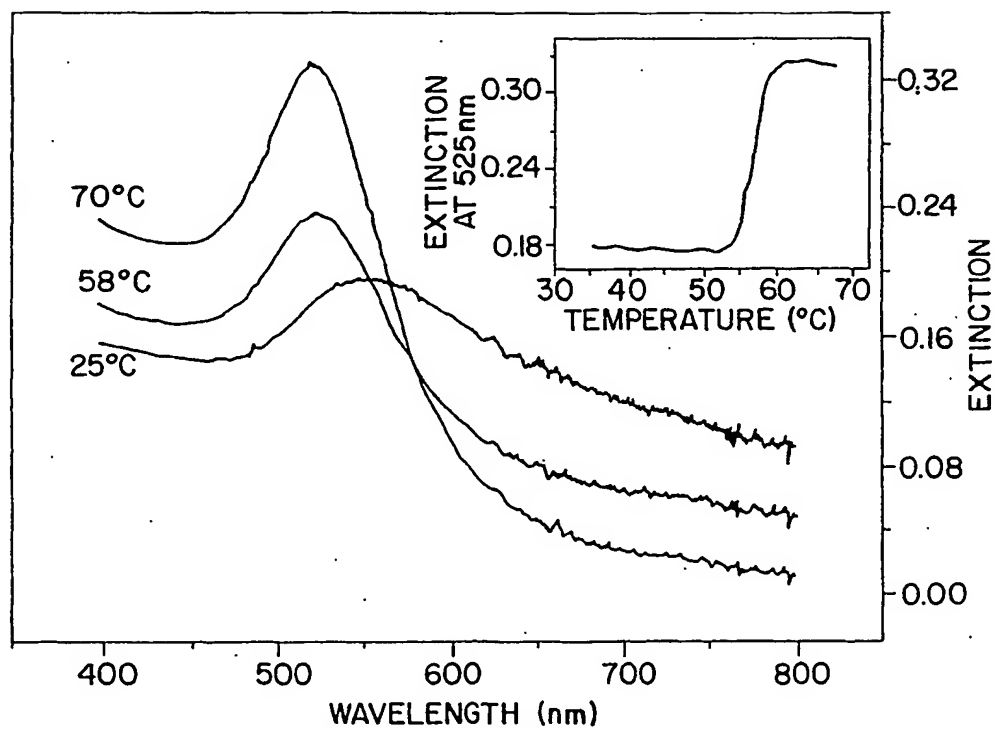


FIG. 28A

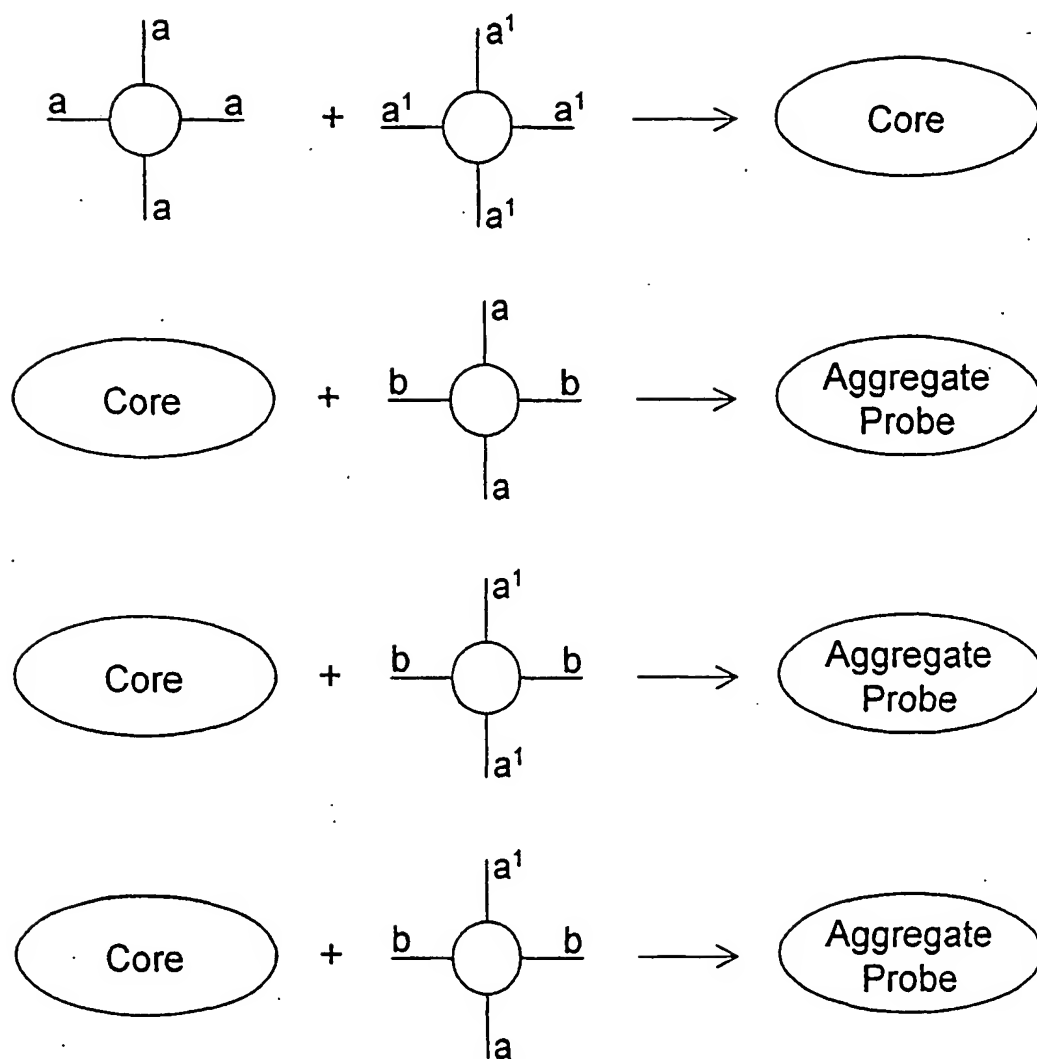




FIG. 28B

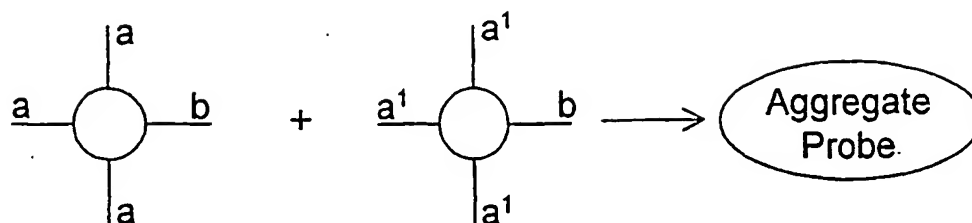


FIG. 28C

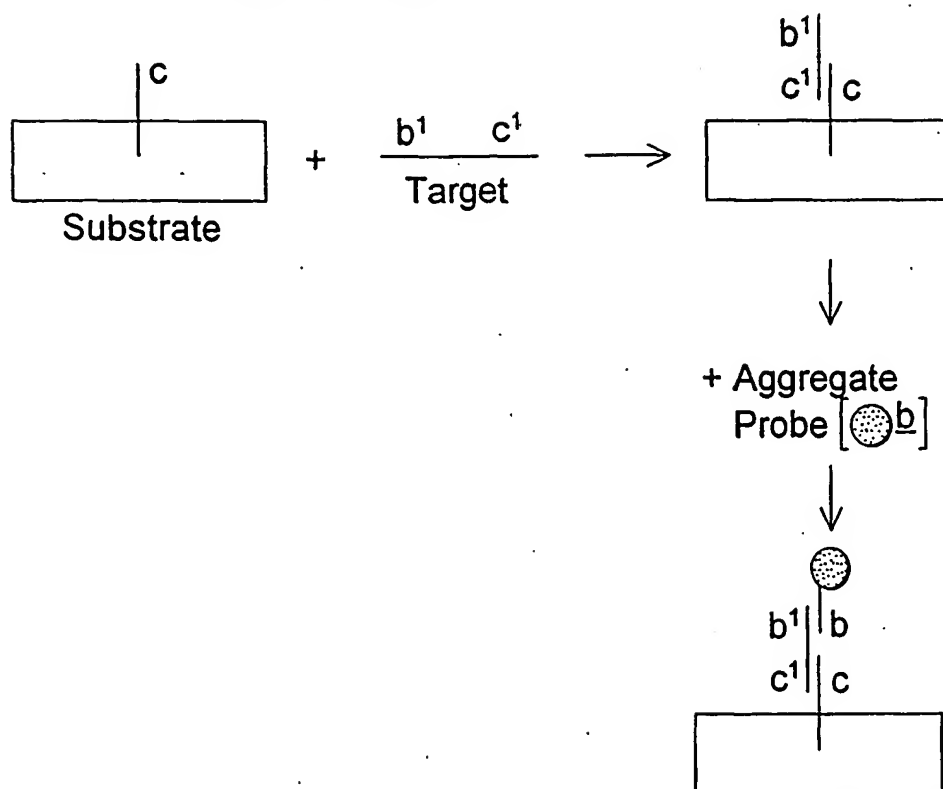


FIG. 28D

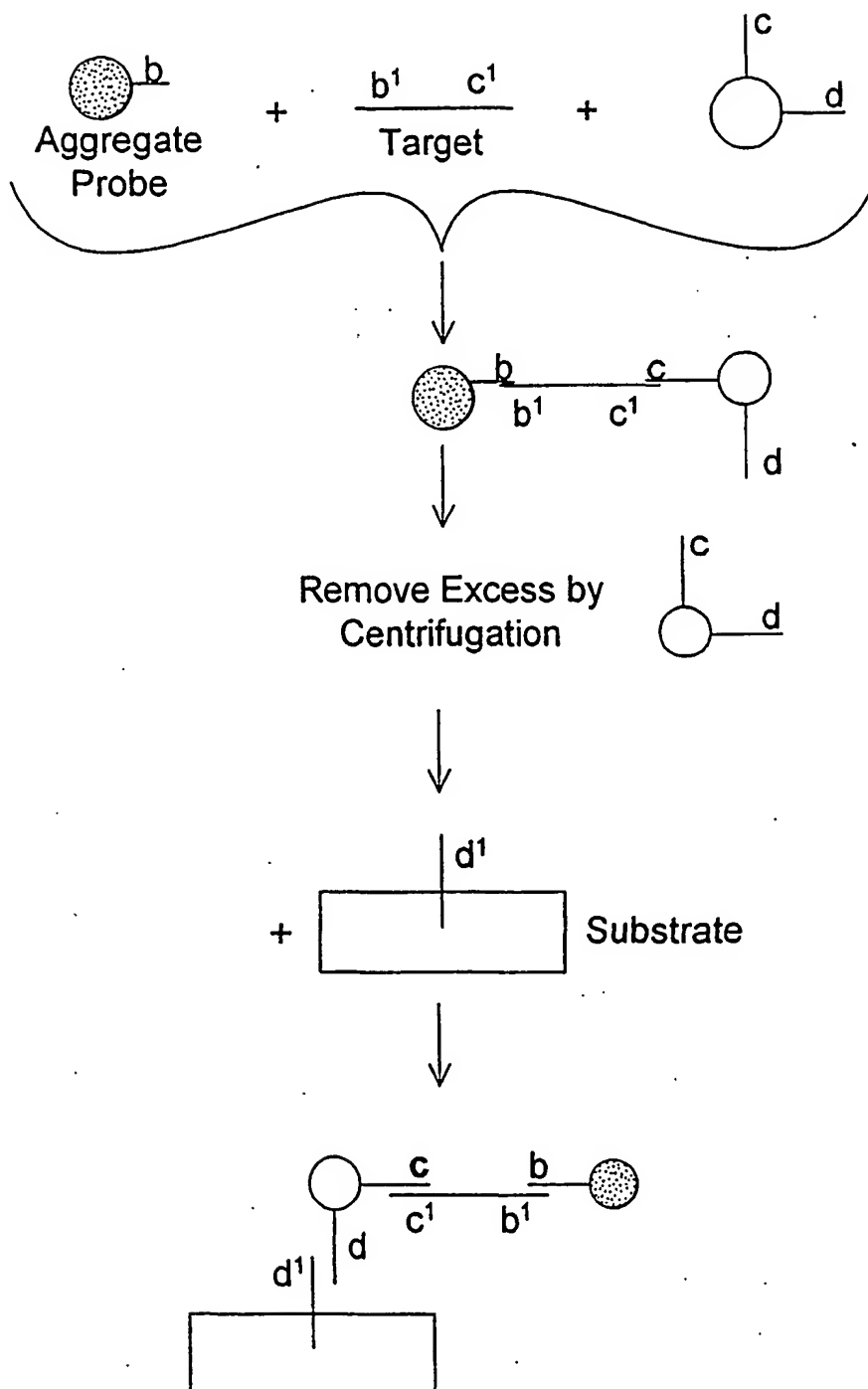
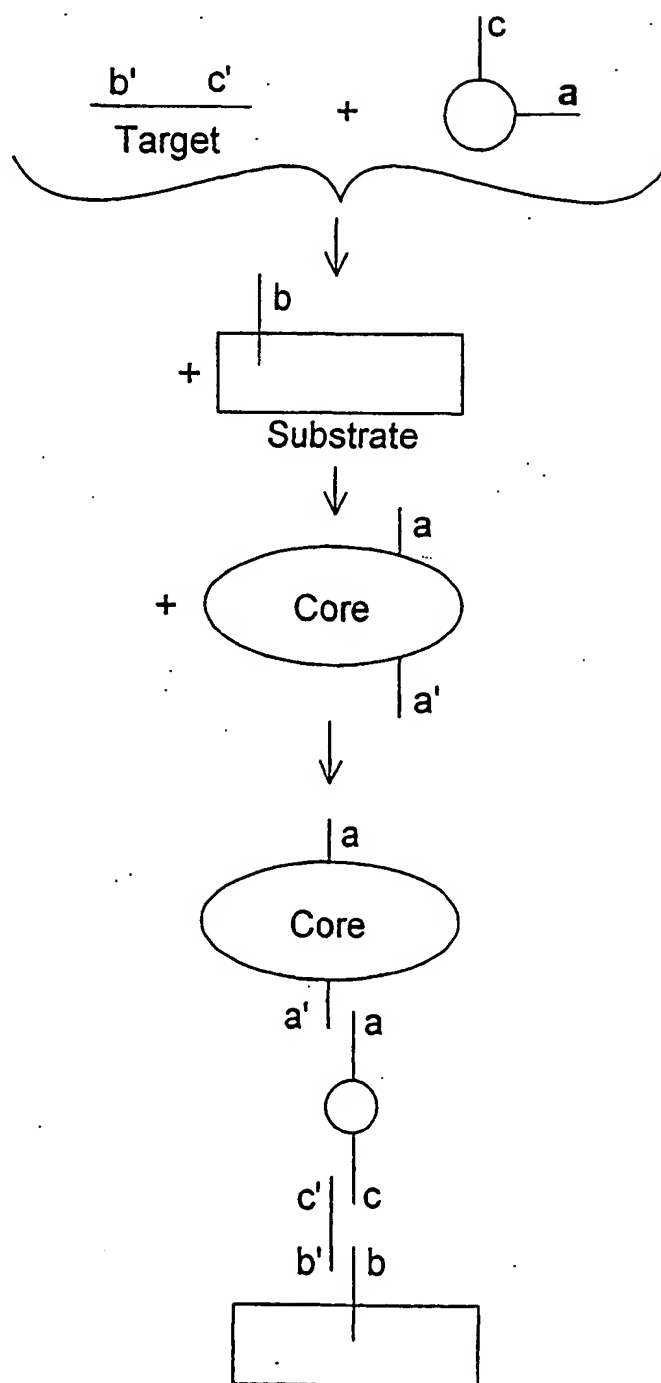


FIG. 28E



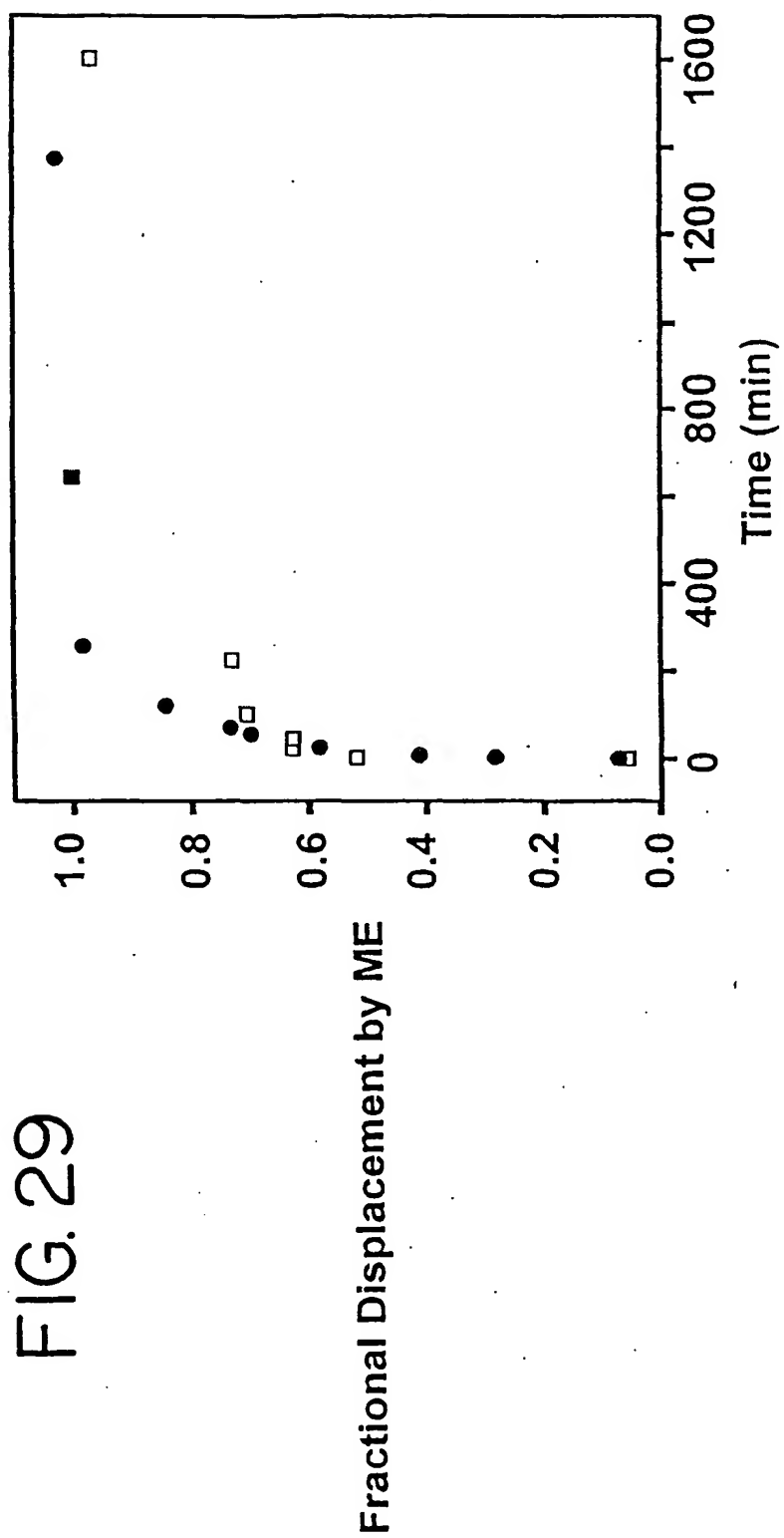


FIG. 30

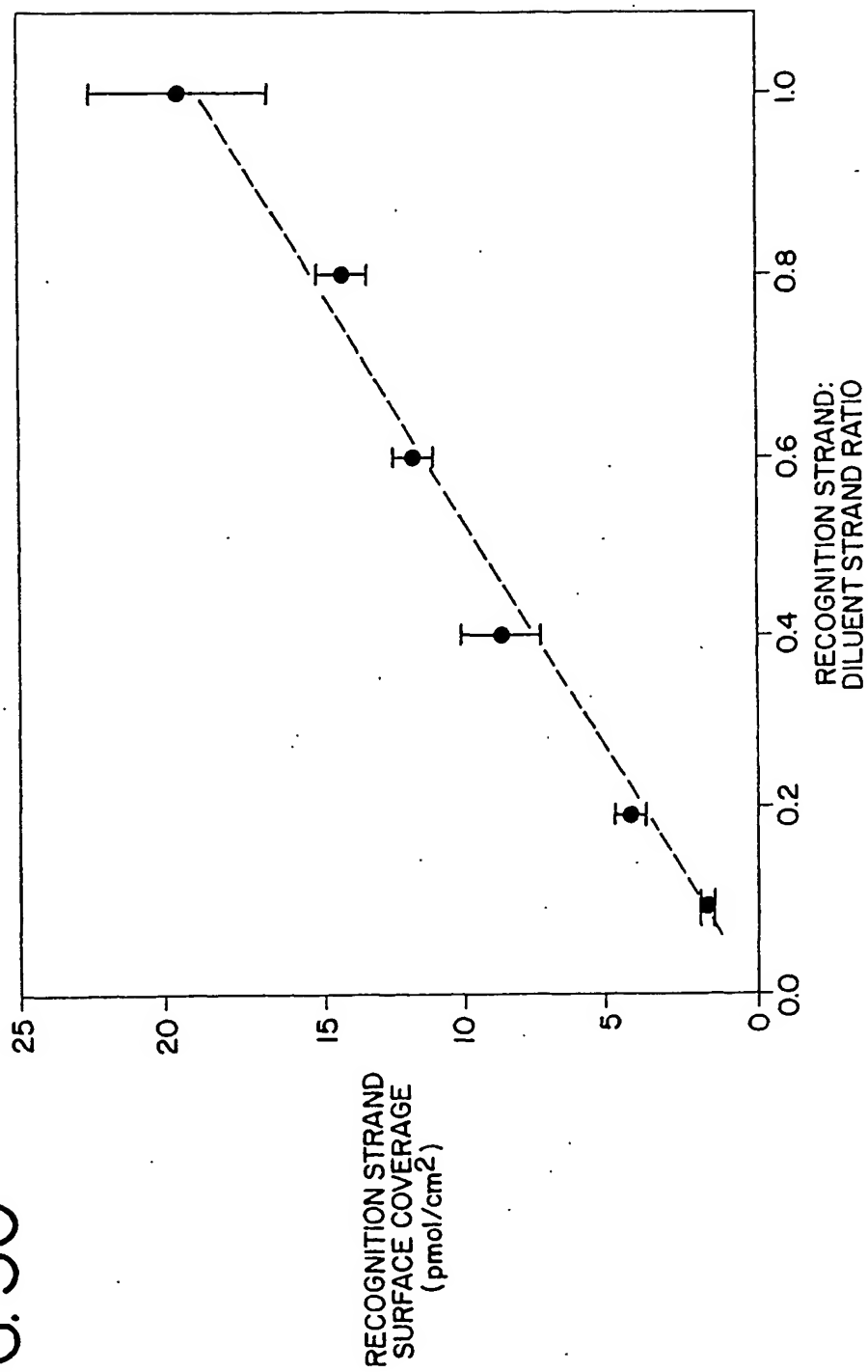


FIG. 31

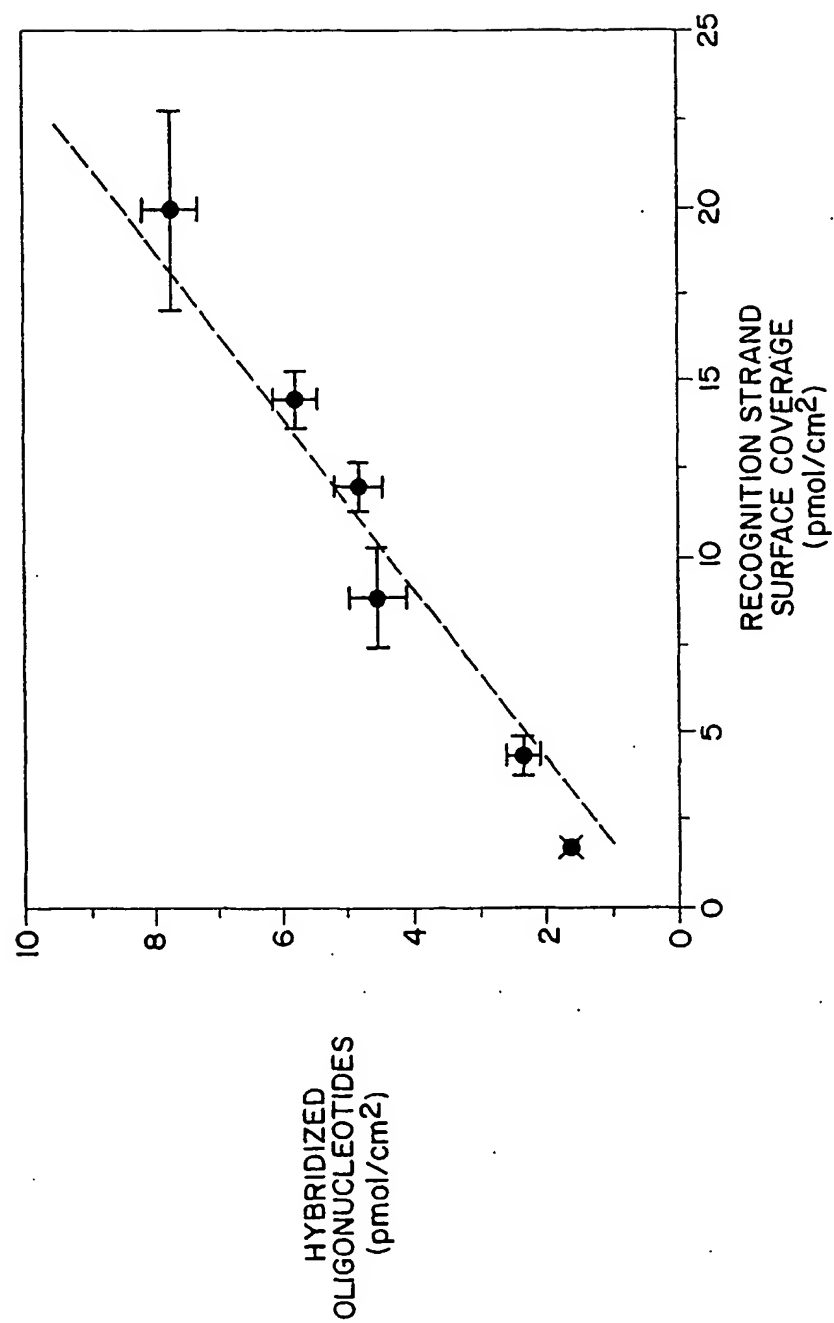
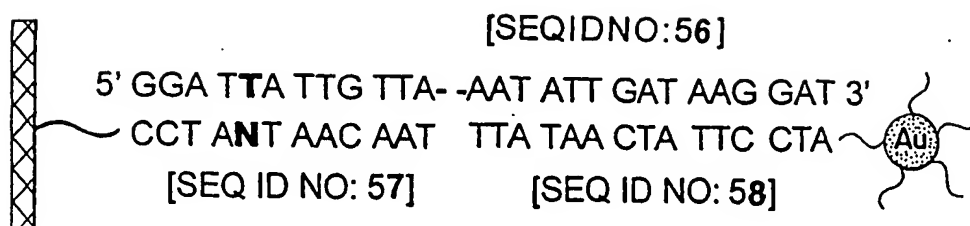


FIG. 32



**N** = A (complementary),  
G,C,T (mismatched)

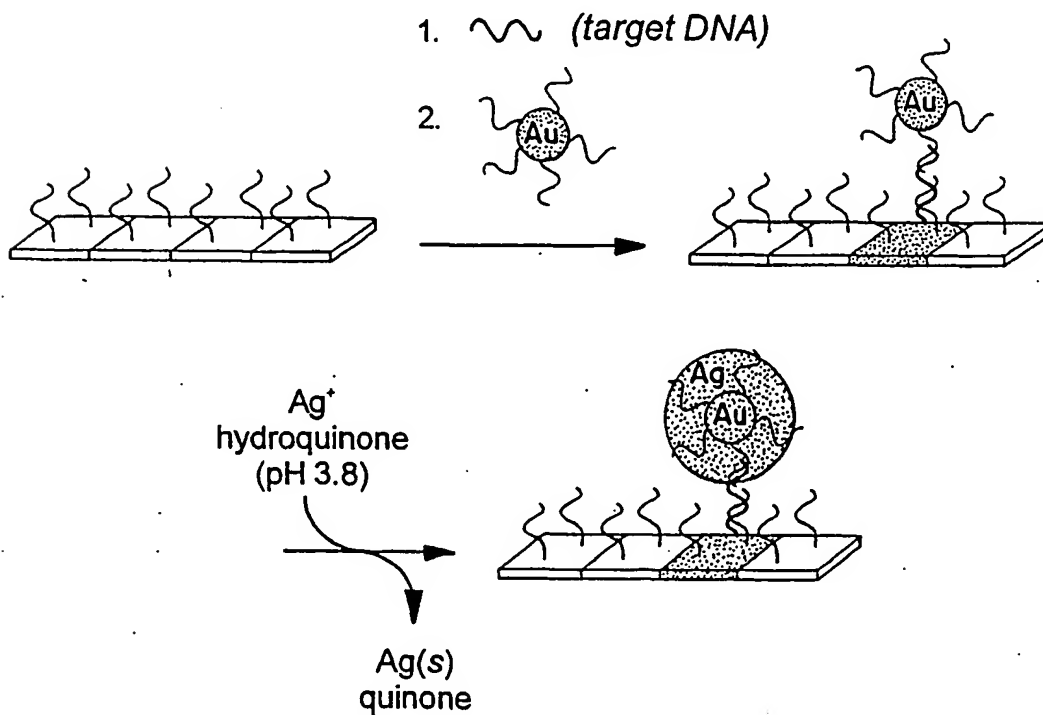


FIG. 33

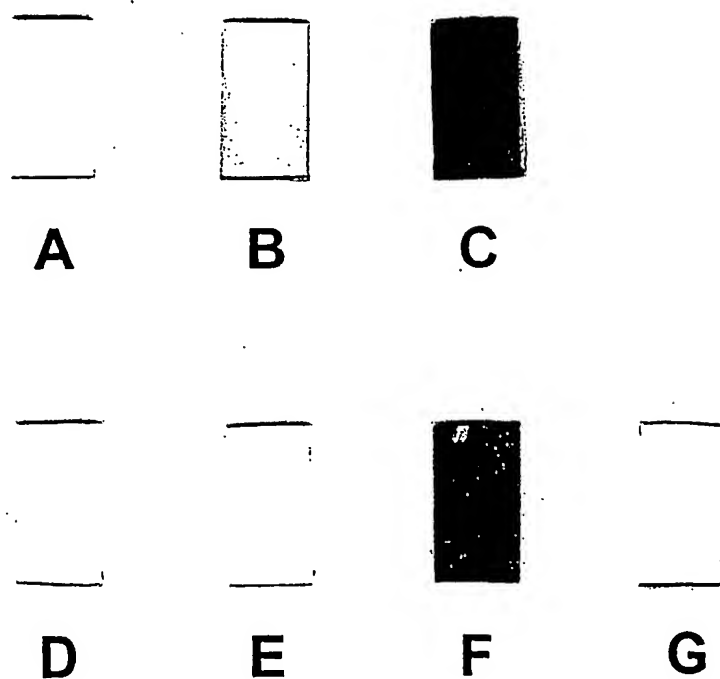




FIG. 34

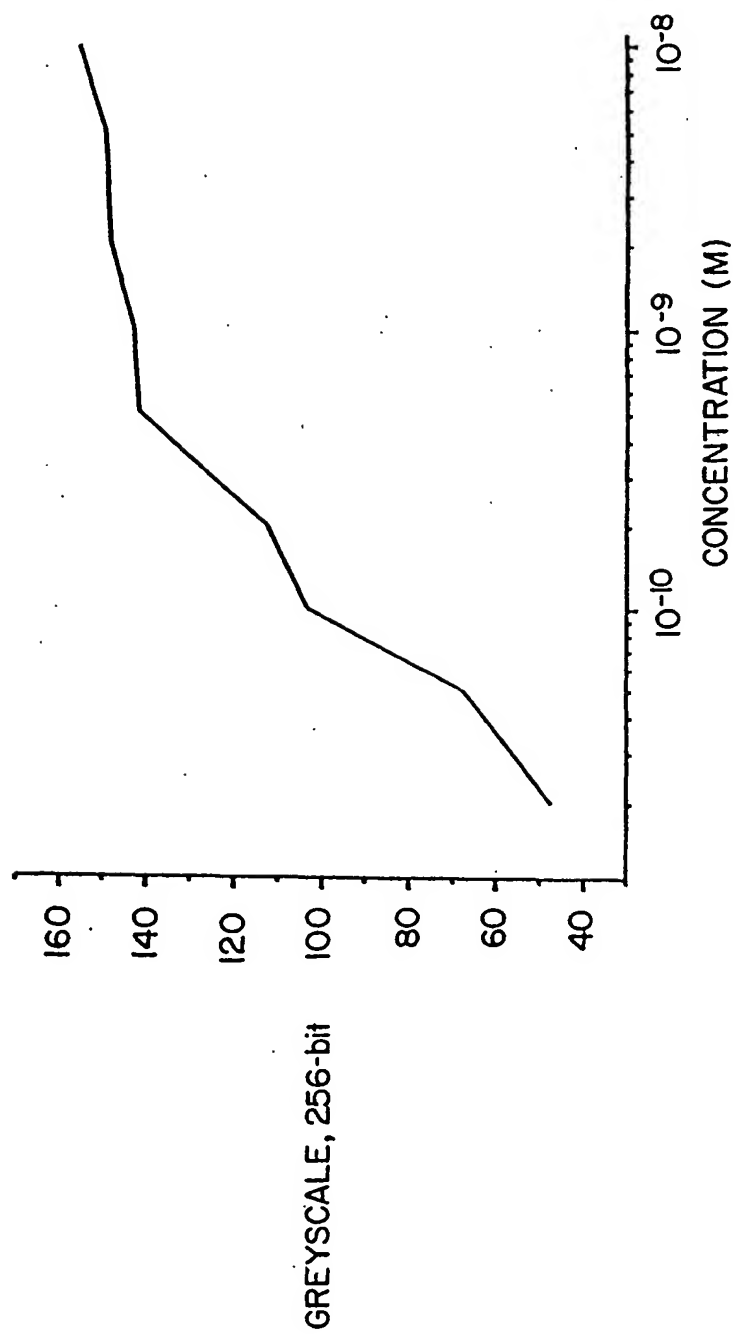


FIG.35A

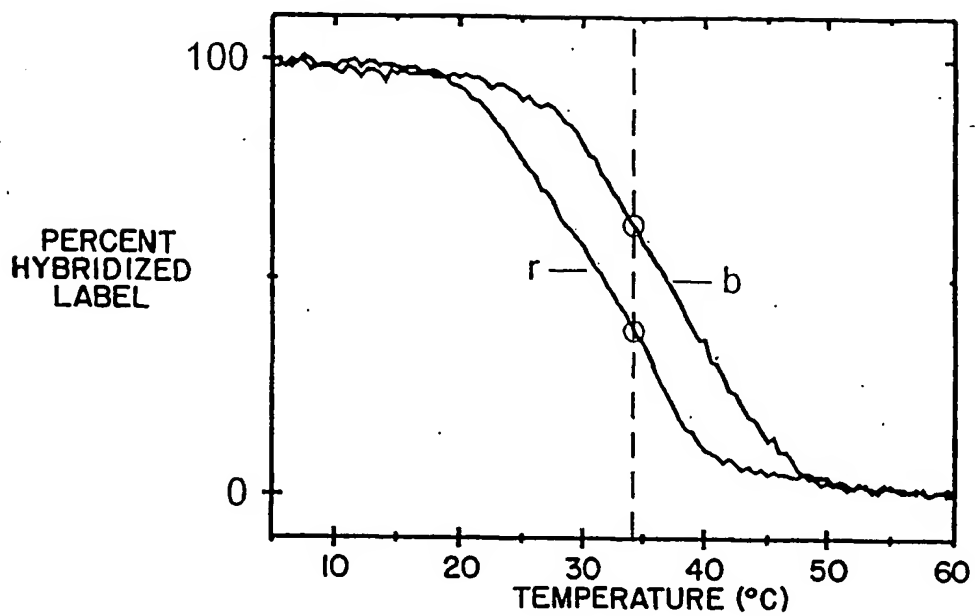


FIG.35B

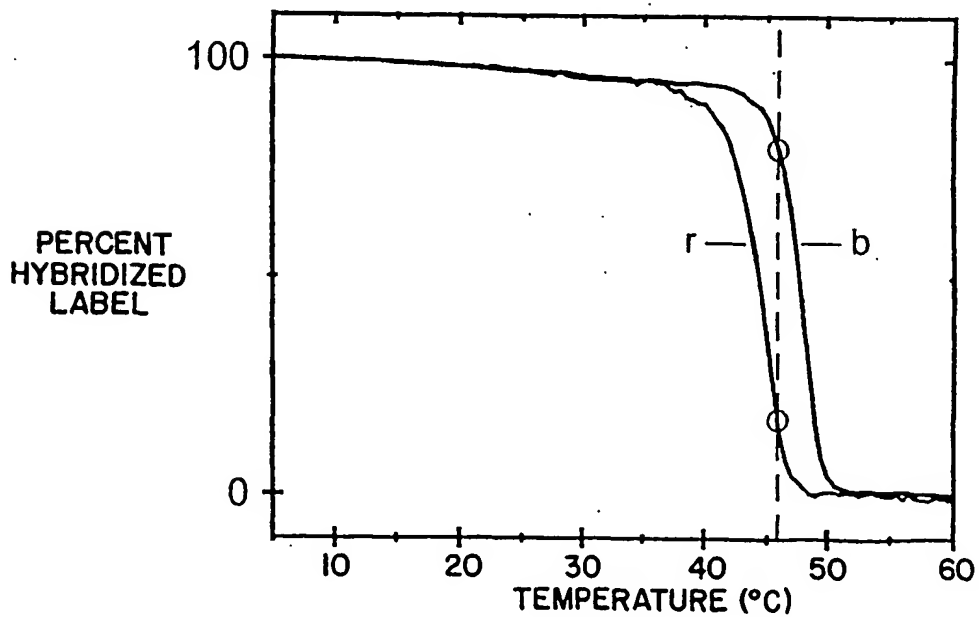


FIG. 36A

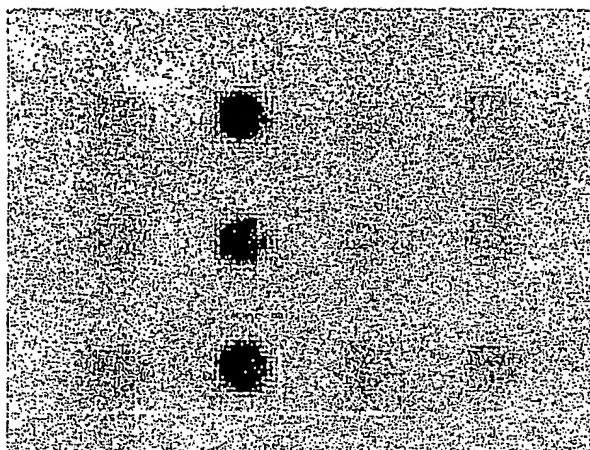
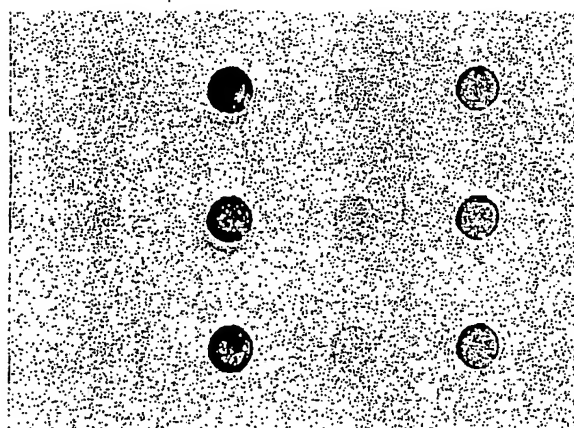


FIG. 36B



C A T G

FIG.37A

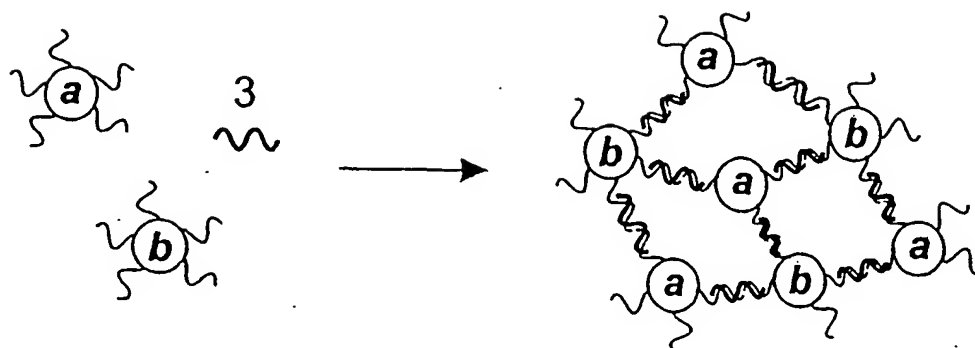


FIG.37B

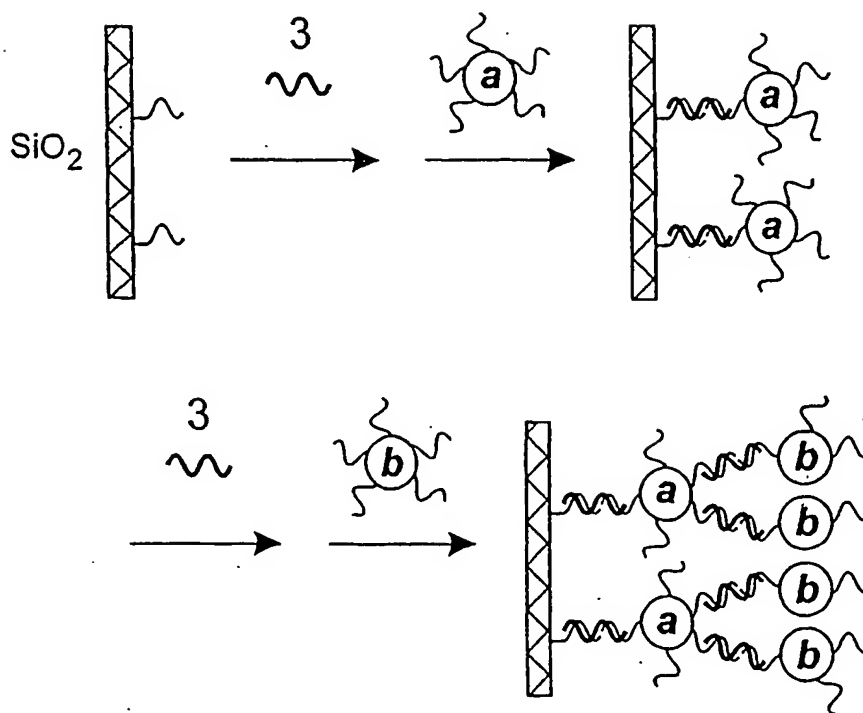


FIG. 38A

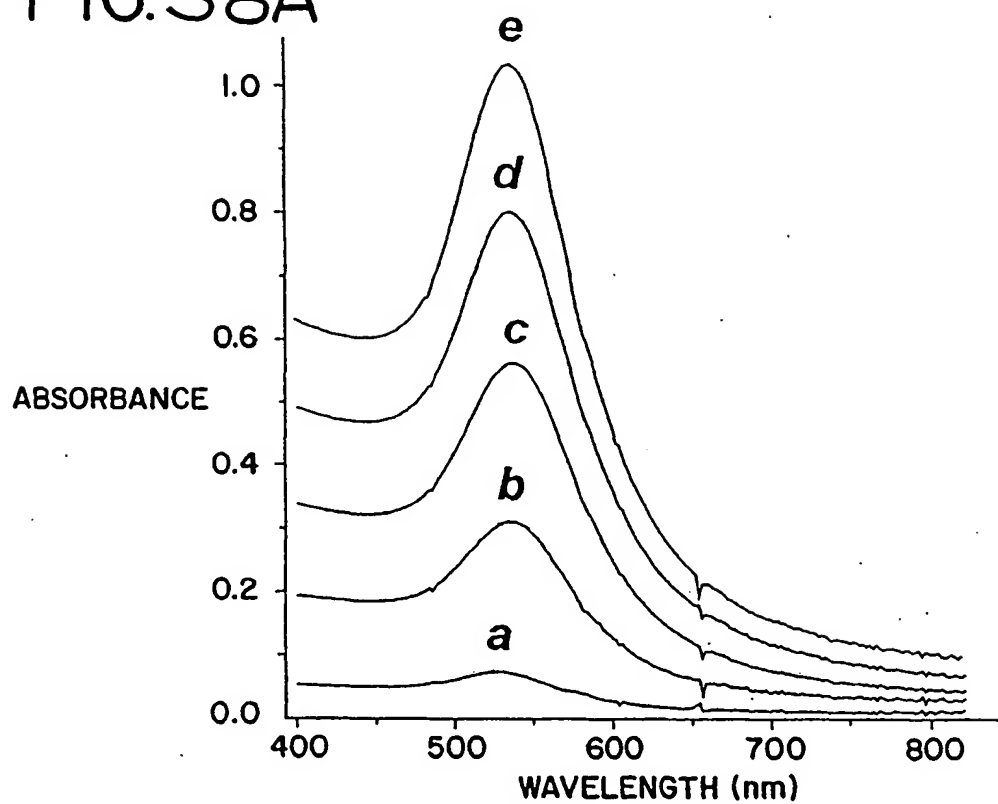


FIG. 38B

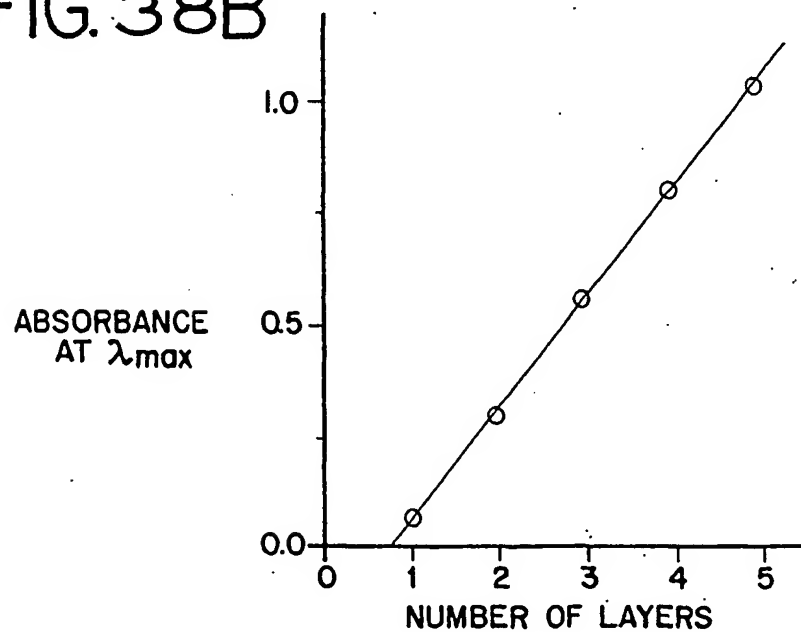


FIG. 39A

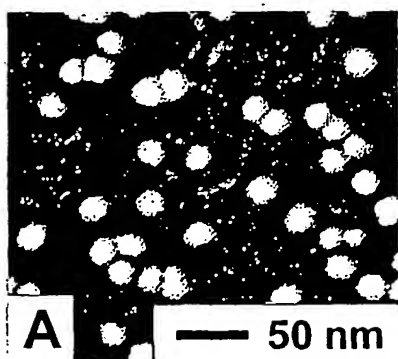


FIG. 39B

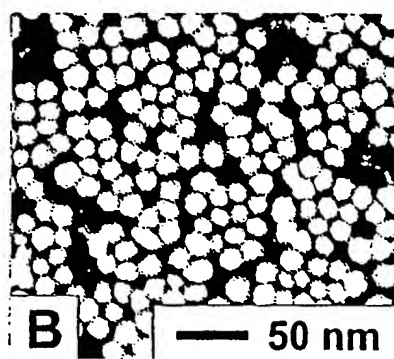


FIG.39C

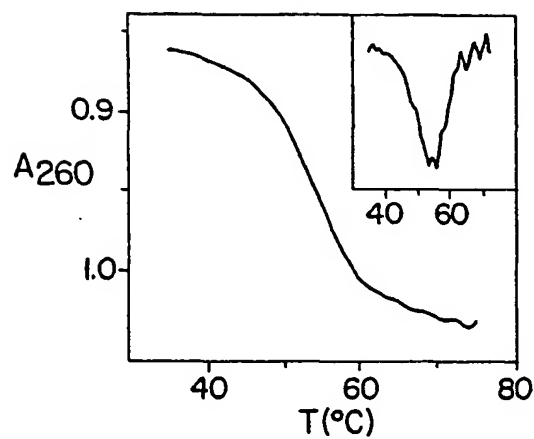


FIG.39D

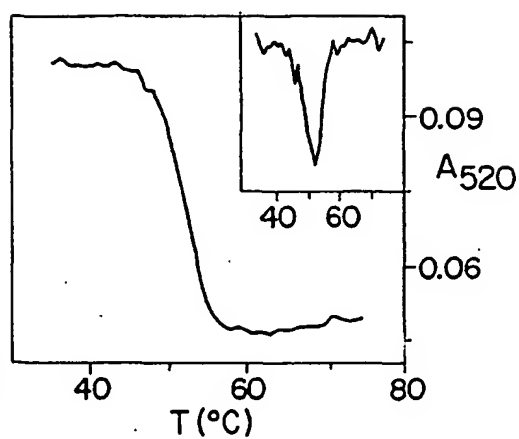


FIG.39E

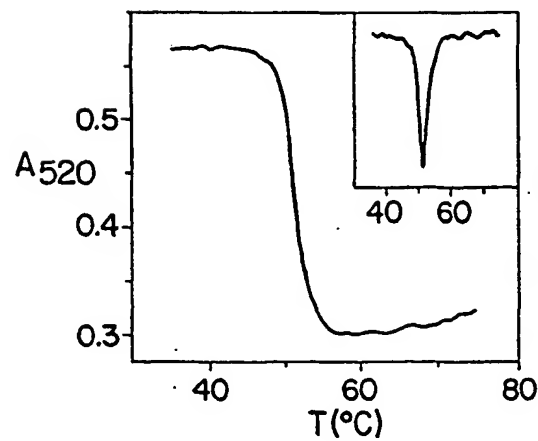


FIG.39F

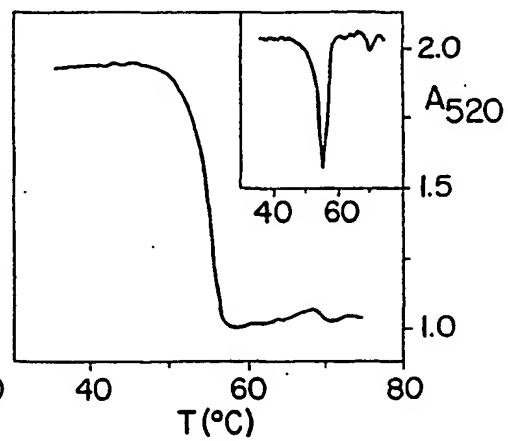


FIG. 40

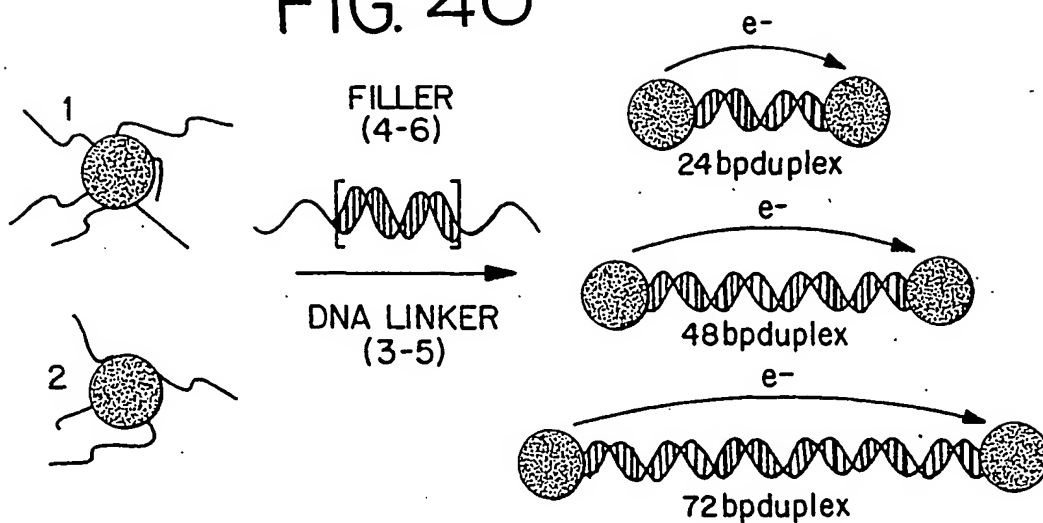
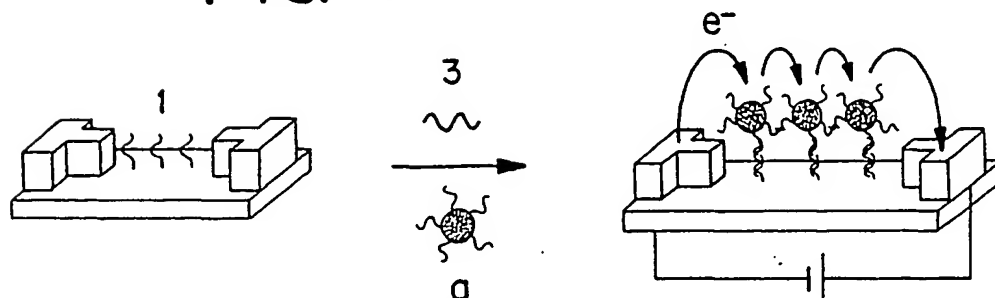
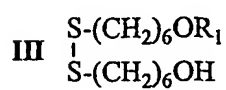
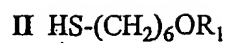
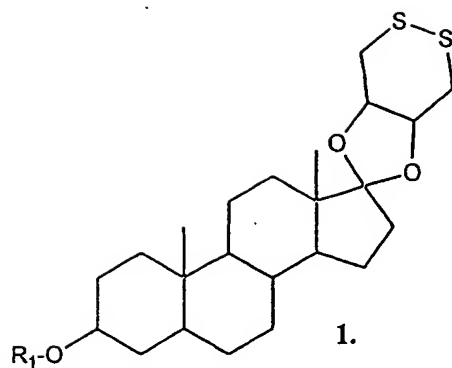


FIG. 41





## FIG. 42



R<sub>1</sub>

a = H

b = (iPr)<sub>2</sub>NP(OCH<sub>2</sub>CH<sub>2</sub>CN)-

c1 = 5'-p(A<sub>20</sub>)-TATCGTTCCATCAGCT [SEQ ID NO: 65]

c2 = 5'-p(A<sub>20</sub>)-TTGATCTTCCGTTCT [SEQ ID NO: 66]

Target I = 79-mer oligonucleotide with target region:

3'-.....ATAGCAAGGTAGTCGAGCAACTAGAAAGGCAAGA.....5'  
[SEQ ID NO: 67]

FIG. 43

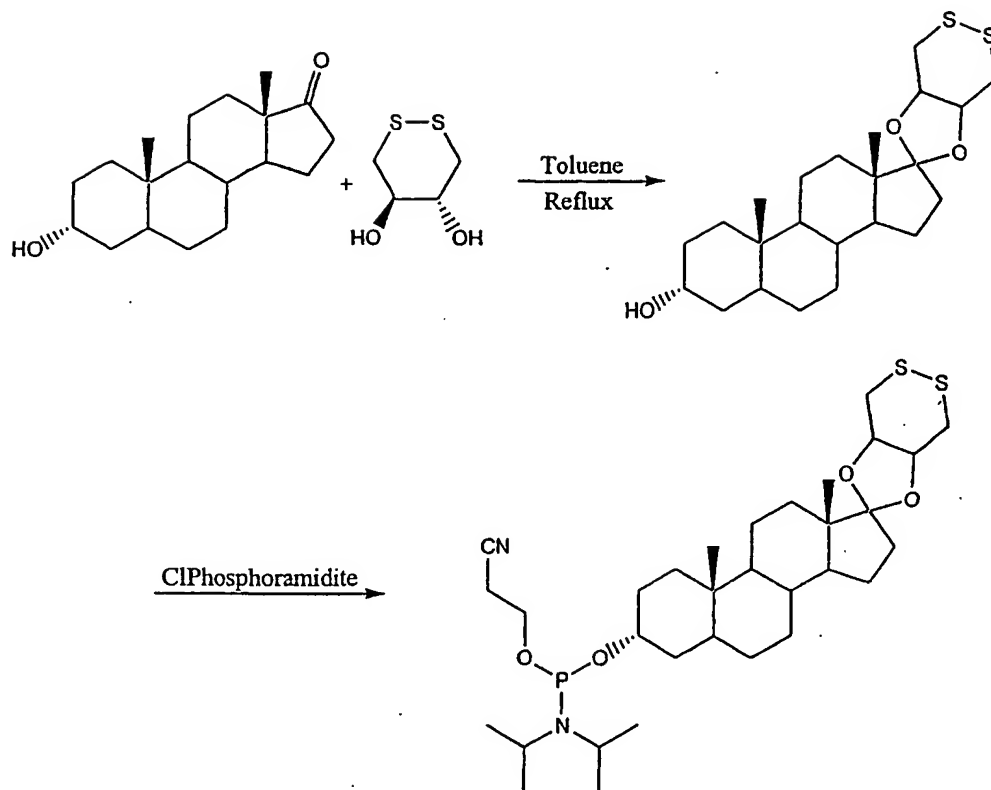
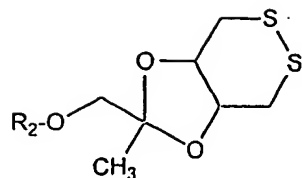


FIG. 44



2.

 $R_2$ 

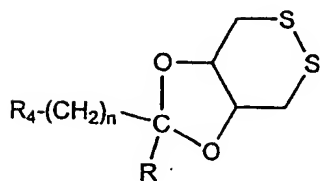
a = H

b = (iPr)<sub>2</sub>NP(OCH<sub>2</sub>CH<sub>2</sub>CN)-c1 = 5'-p(A<sub>20</sub>)-GCAGACCTCA [SEQ ID NO: 68]c2 = 5'-p(A<sub>20</sub>)-CCTATGTGTCG [SEQ ID NO: 69]D = 5'-p(A<sub>20</sub>) [SEQ ID NO: 70]

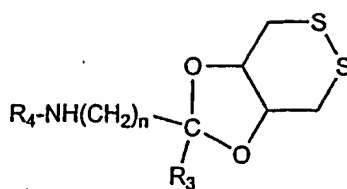
Target I = 63-mer oligonucleotide with target region:

3'-.....CGTCTGGAGTGGATACACAGC.....5'

[SEQ ID NO: 71]



3.



4.

 $R_3$  = hydrogen, an alkyl group, an aryl group, or a substituted alkyl or aryl group $R_4$  = an attached oligonucleotide or modified oligonucleotide

FIG. 45

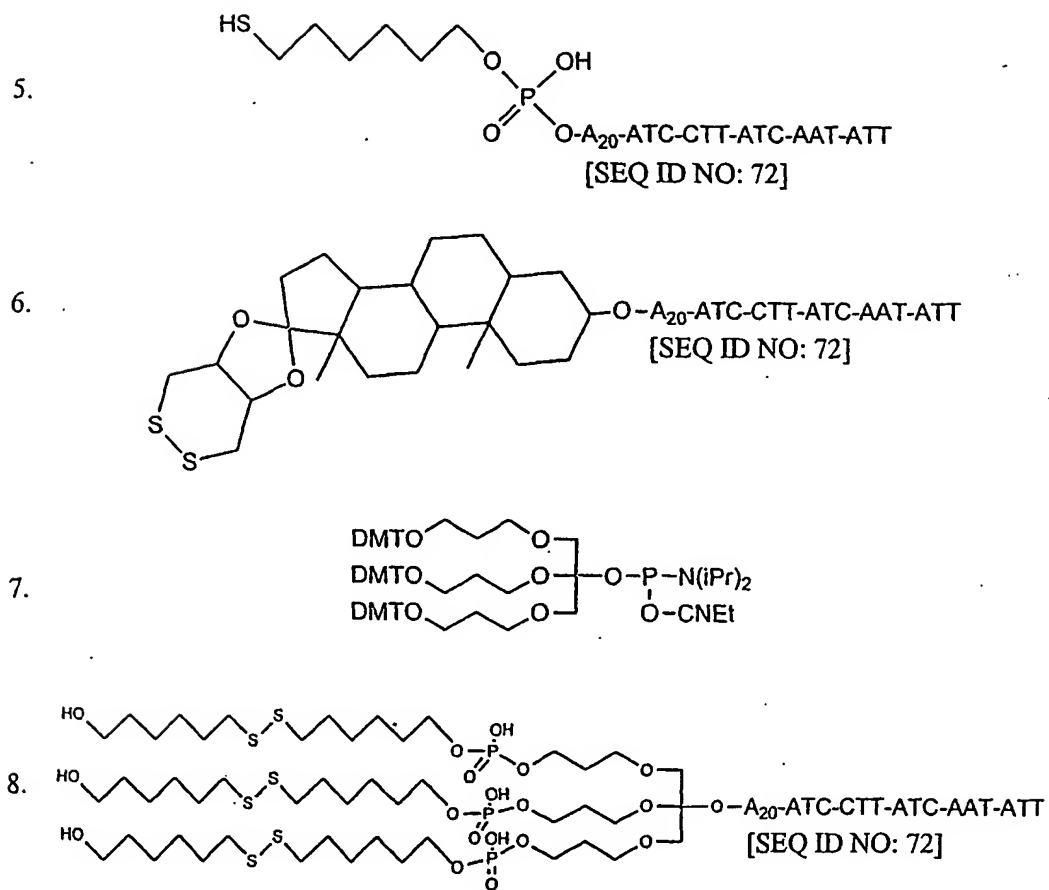
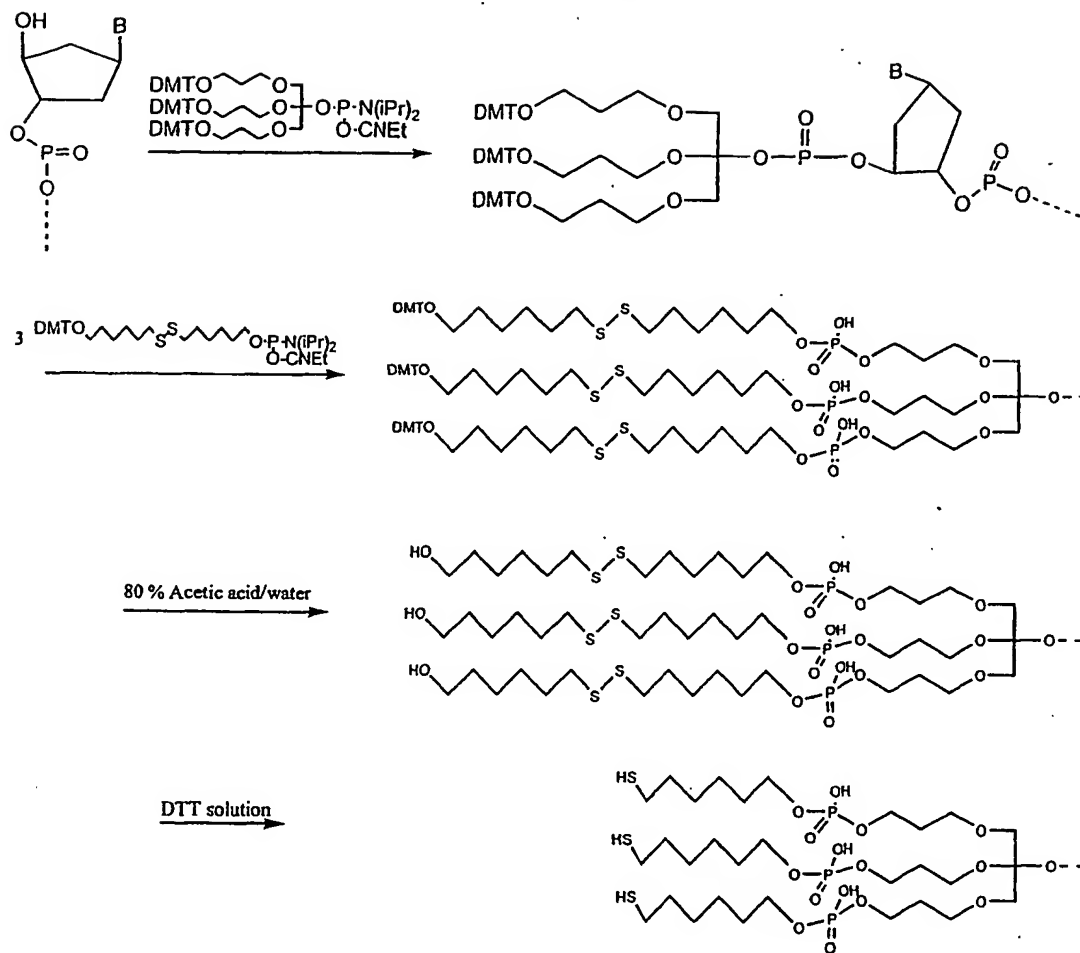


FIG. 46



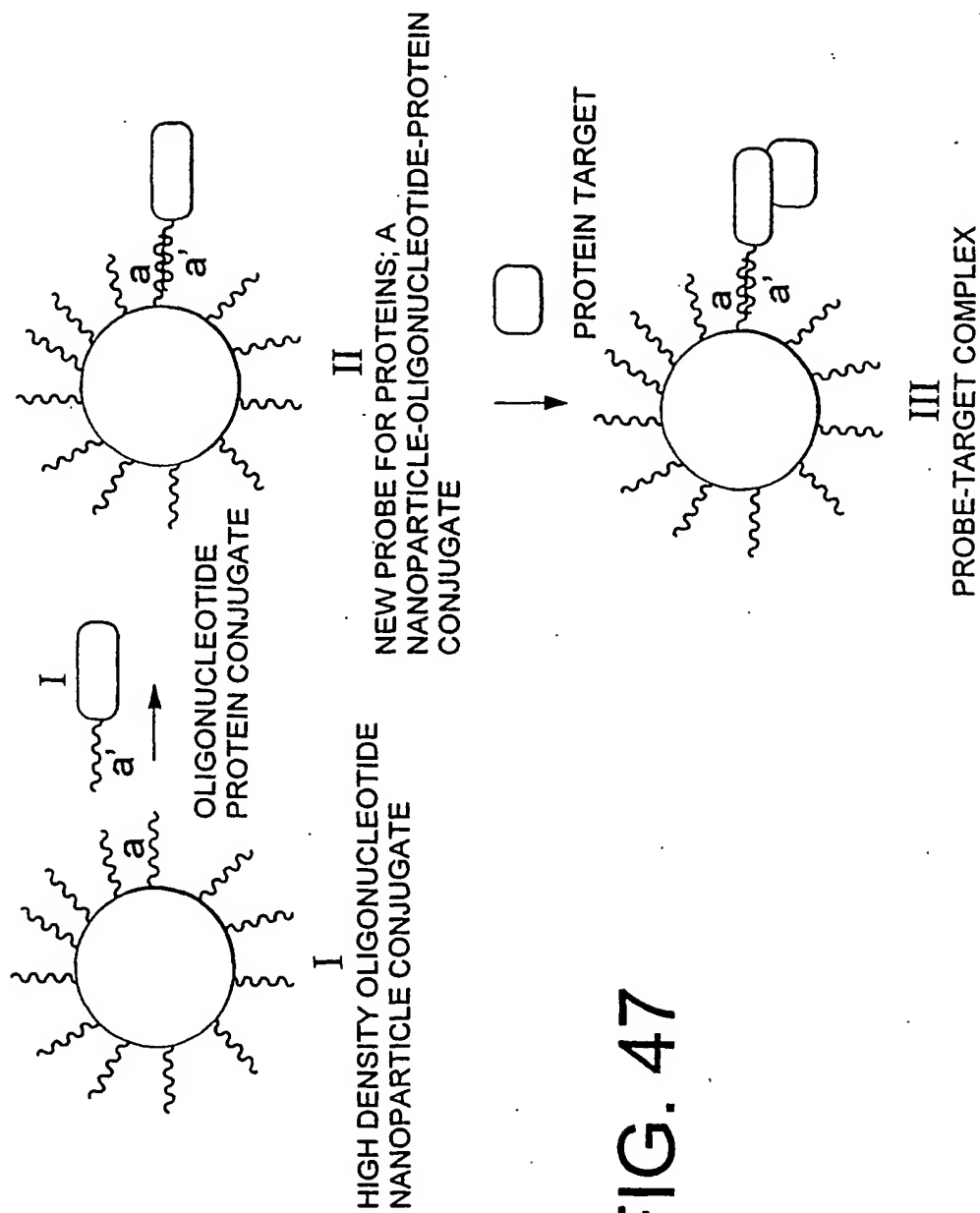
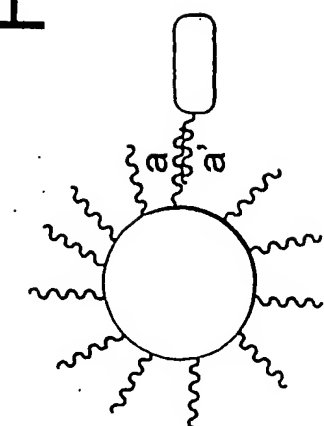


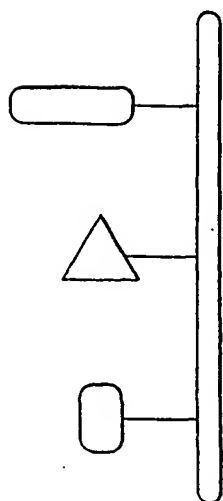
FIG. 47

FIG. 48



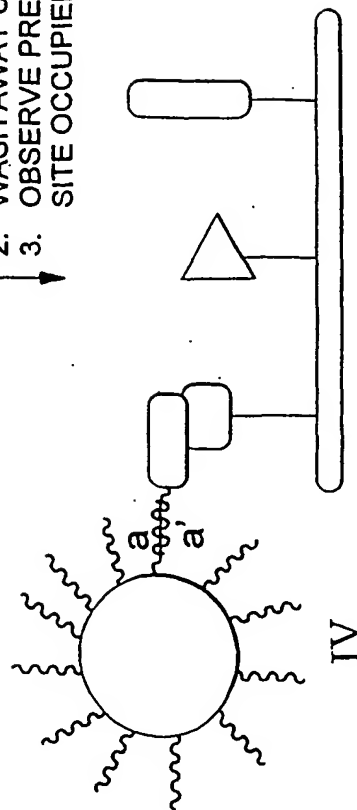
II

NEW PROBE FOR PROTEINS; A  
NANOPARTICLE-OLIGONUCLEOTIDE-PROTEIN  
CONJUGATE

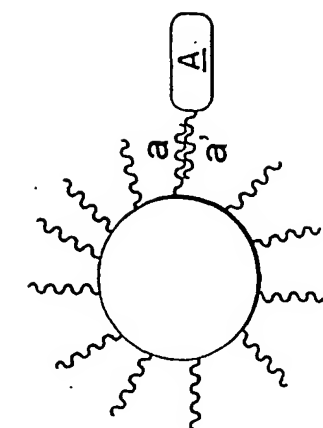


GLASS PLATE WITH THREE DIFFERENT PROTEINS  
IMMOBILIZED ON THE SURFACE, ONE OF WHICH  
BINDS TO THE PROTEIN IN PROBE II

1. EXPOSE PLATE TO THE PROBE SOLUTION
2. WASH AWAY UNBOUND NANOPARTICLE PROBE
3. OBSERVE PRESENCE OF BOUND NANOPARTICLES AT  
SITE OCCUPIED BY THE FIRST PROTEIN IN THE SERIES.

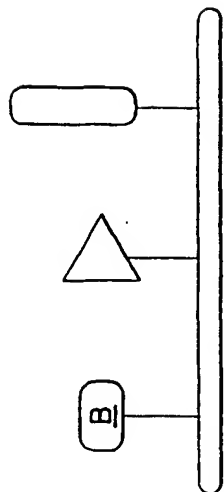


IV



II'

NANOPARTICLE-OLIGONUCLEOTIDE-RECEPTOR



GLASS PLATE WITH THREE DIFFERENT SUBSTANCES  
IMMOBILIZED ON THE SURFACE, ONE OF WHICH (B)  
BINDS TO THE RECEPTOR UNIT (A) IN II'.

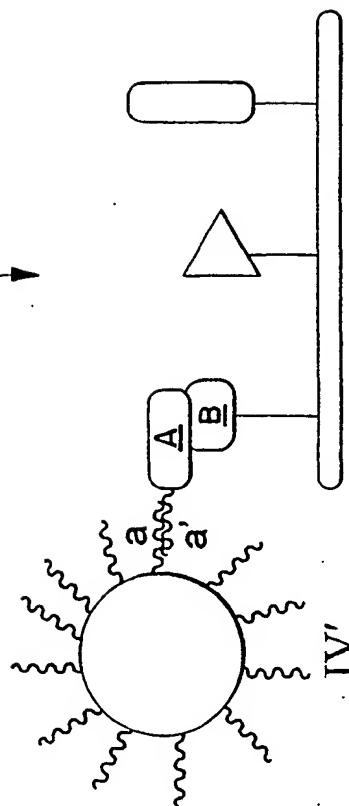
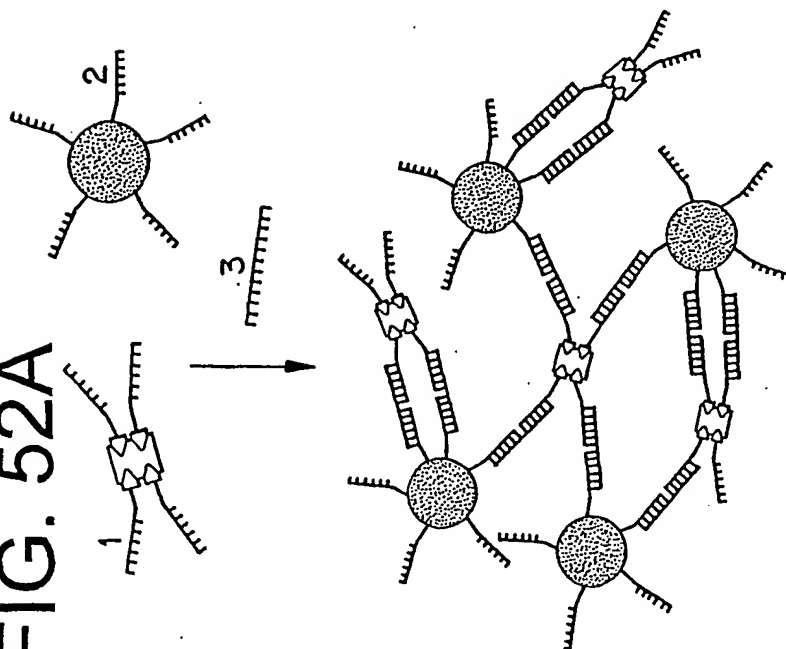


FIG. 49



A diagram of a cell with a central, shaded, circular nucleus. Four flagella extend from the nucleus. Each flagellum consists of a short, thick, wavy base (basal body) and a long, thin, whip-like extension (filament). The filaments are shown in various positions, suggesting movement. At the end of each filament is a small, semi-circular, shaded structure, likely a flagellar cap or motor.

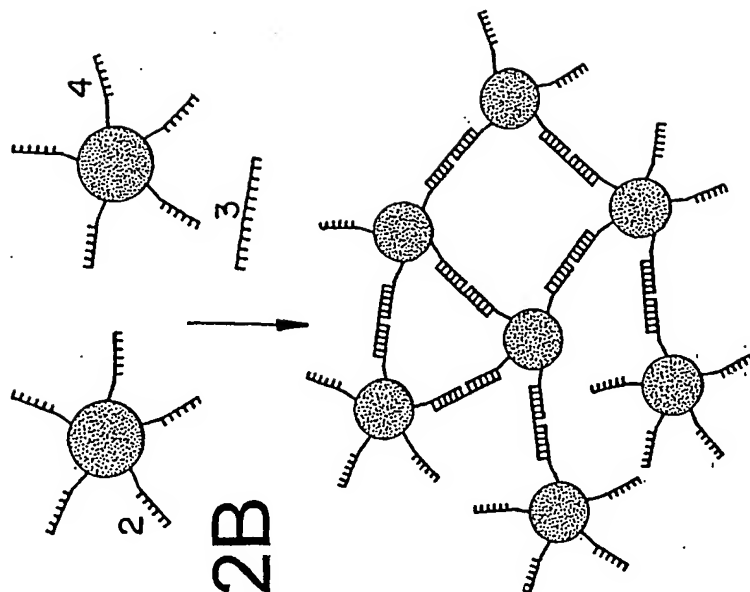
FIG. 52A



- 1 3' biotin-TEG-A<sub>10</sub>-ATG CTC AAC TCT 5' [SEQ. ID NO. 73]  
 2 5' SH(CH<sub>2</sub>)<sub>6</sub>-A<sub>10</sub>-CGC ATT CAG GAT 3' [SEQ. ID NO. 74]  
 3 5' TAC GAG TTG AGA ATC CTG AAT GCG 3' [SEQ. ID NO. 75]

● 13 nm Au NANOPARTICLES    ⌘ STREPTAVIDIN

FIG. 52B



- 2 5' SH(CH<sub>2</sub>)<sub>6</sub>-A<sub>10</sub>-CGC ATT CAG GAT 3'  
 3 5' TAC GAG TTG AGA ATC CTG AAT GCG 3'  
 4 3' SH(CH<sub>2</sub>)<sub>3</sub>-A<sub>10</sub>-ATG CTC AAC TCT 5'

● 13 nm Au NANOPARTICLES

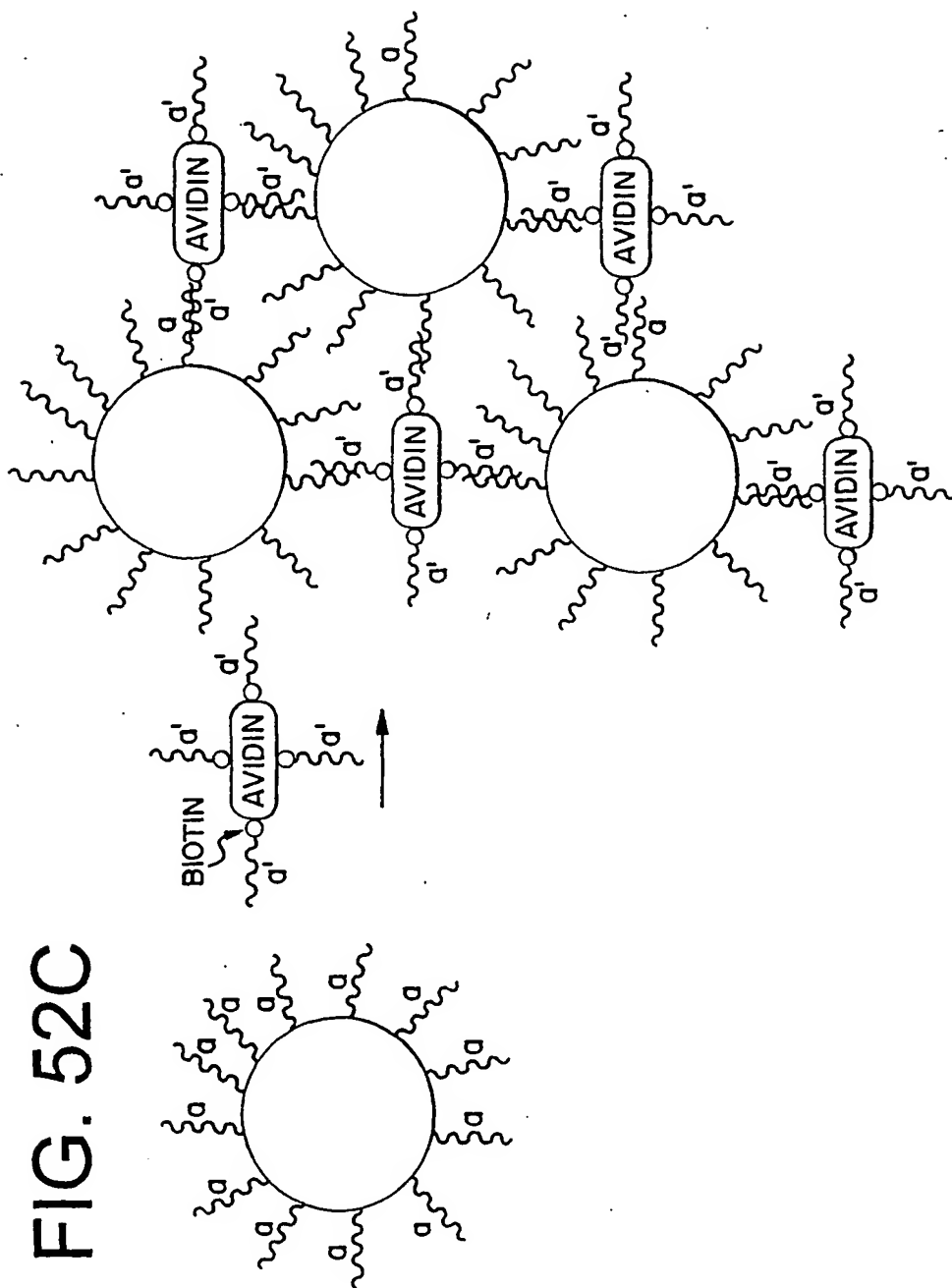


FIG. 52C

FIG. 53

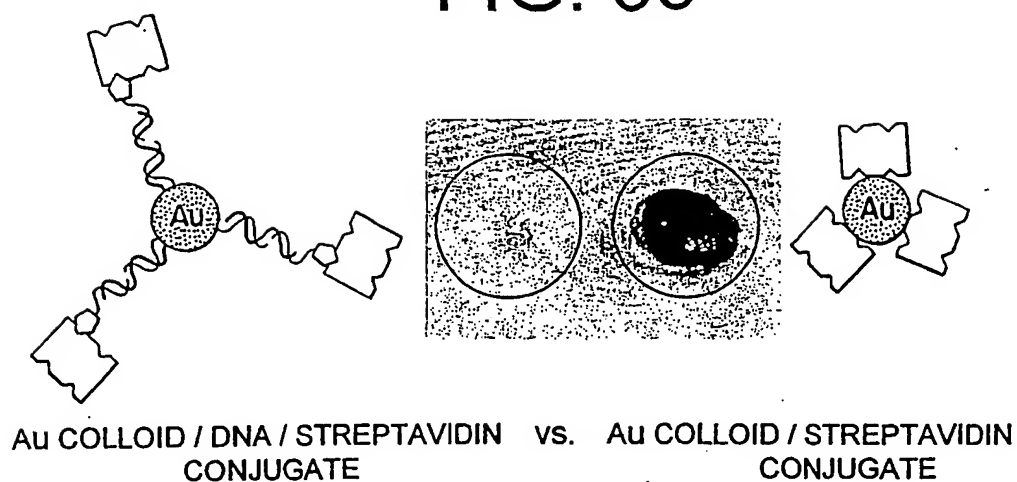


FIG. 54

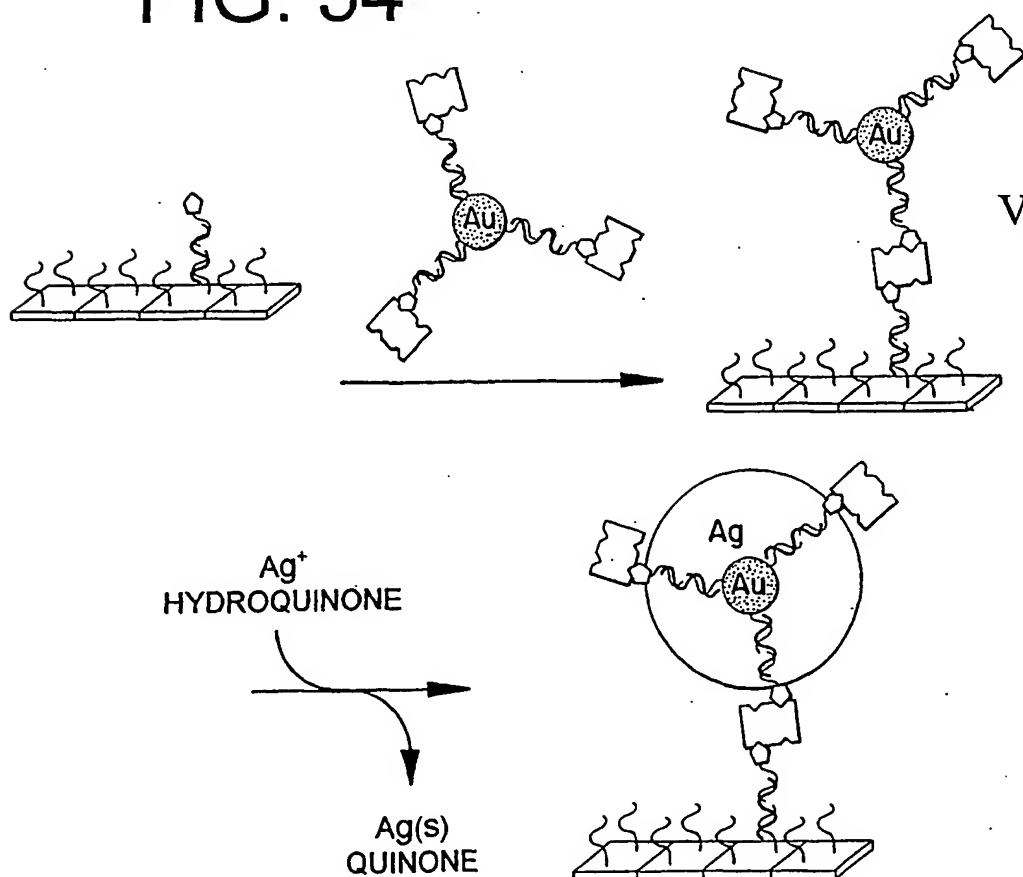


FIG. 58A

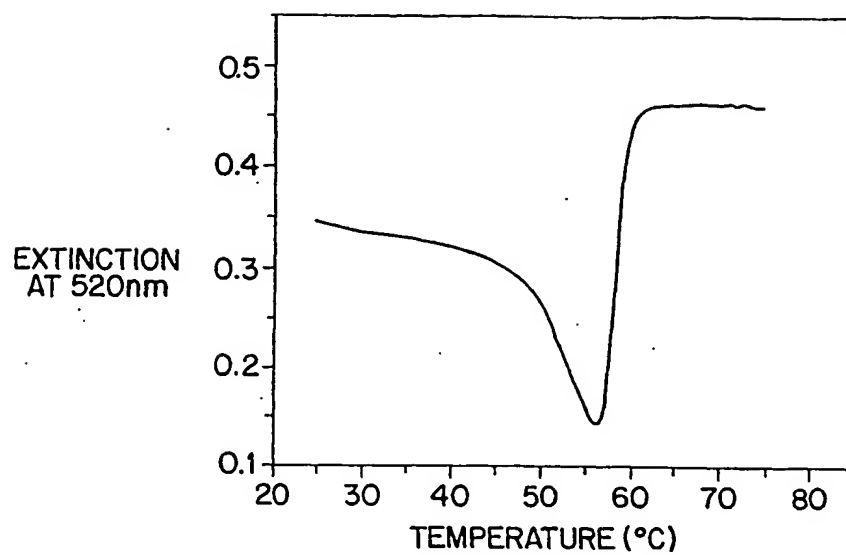


FIG. 58B

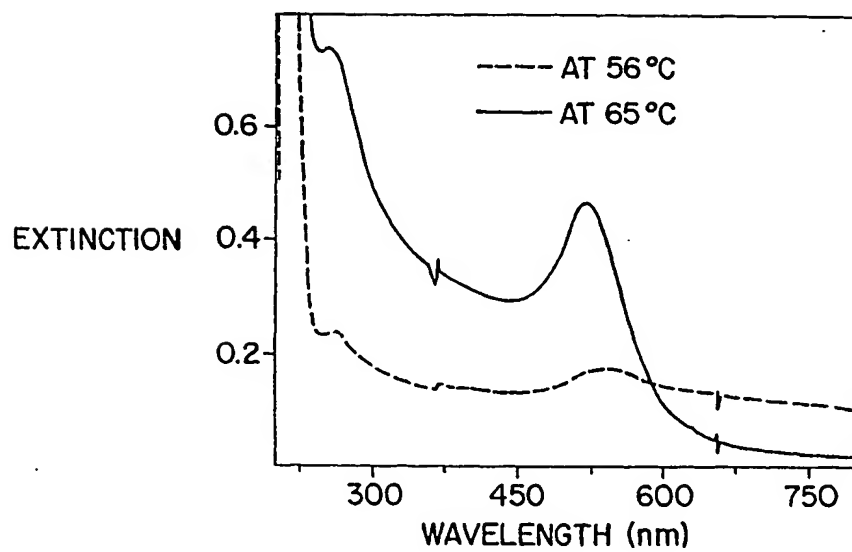


FIG. 59

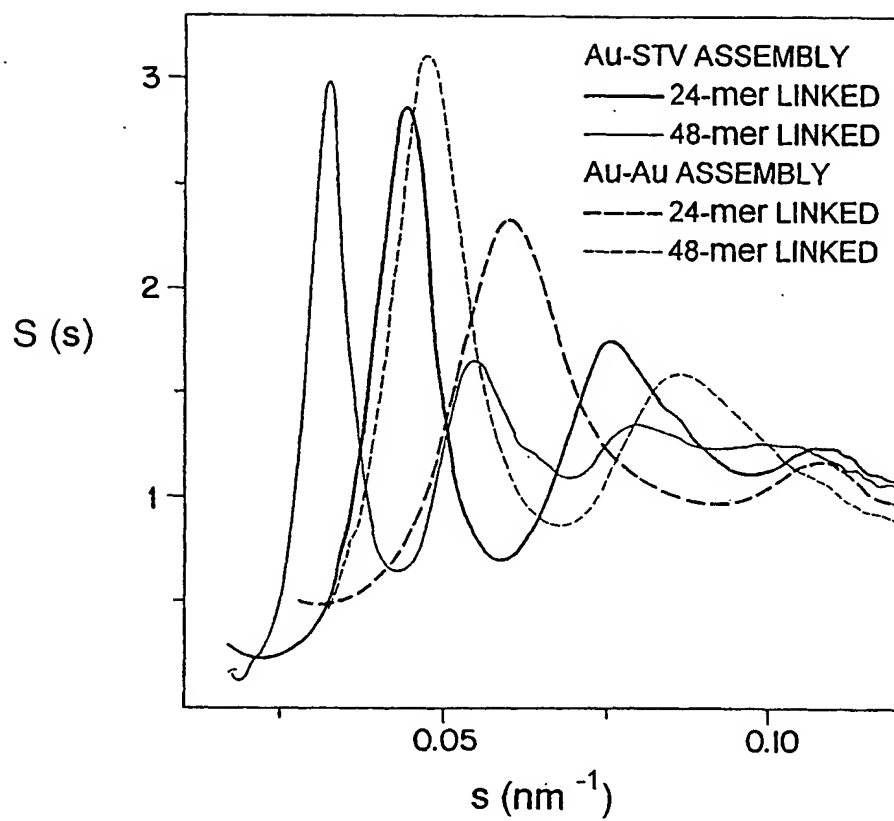


FIG. 60

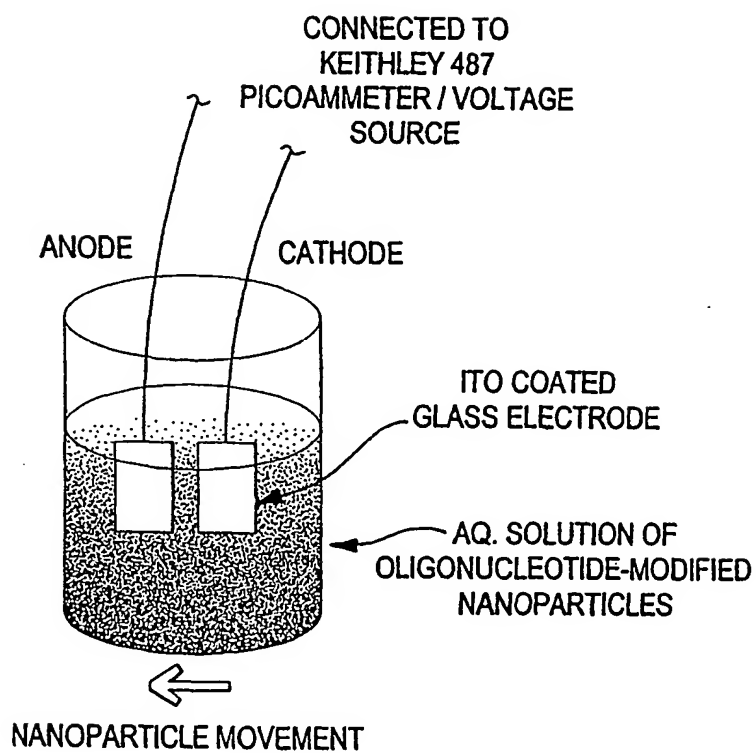
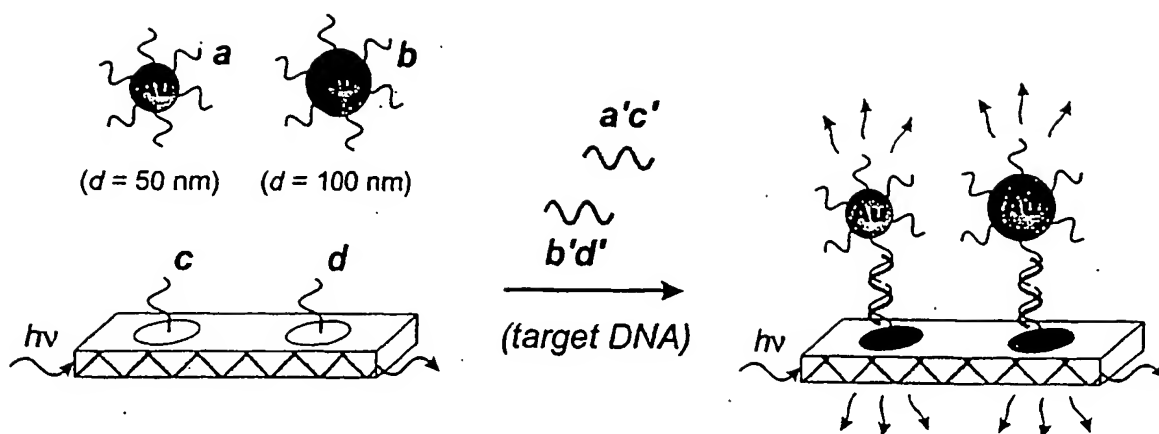
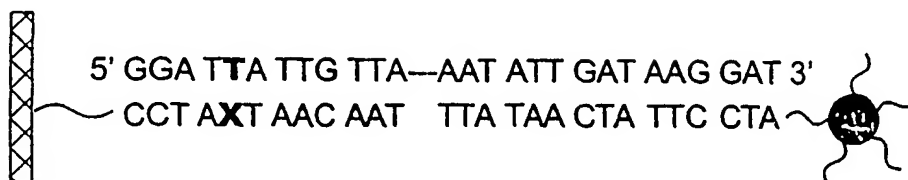


Figure 6)

**A****B**

**X** = A (complementary),  
G,C,T (mismatched)



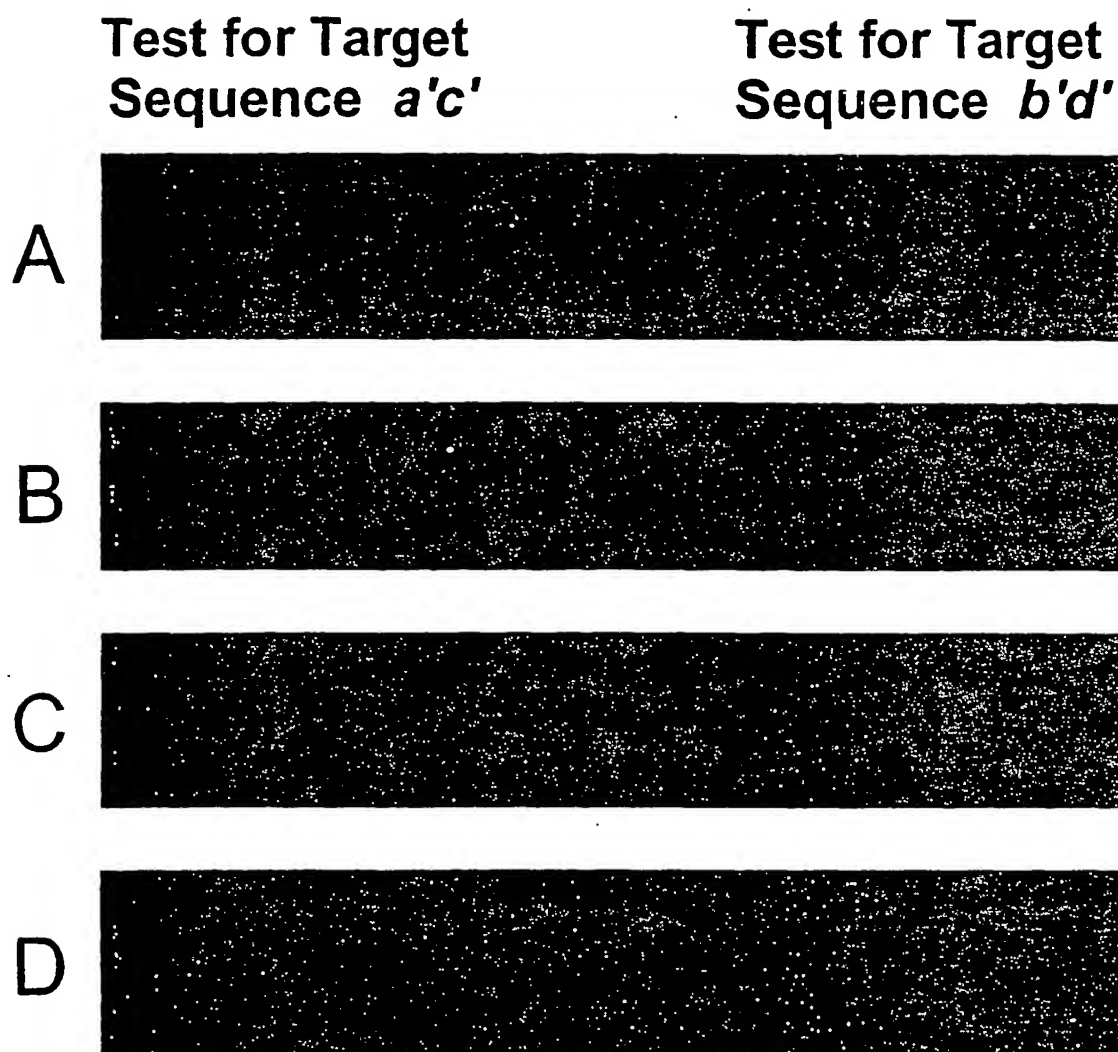
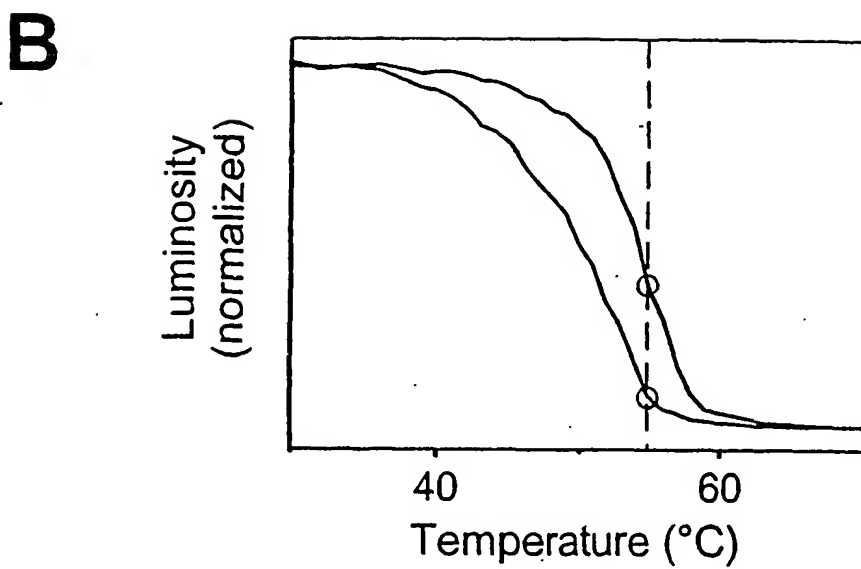
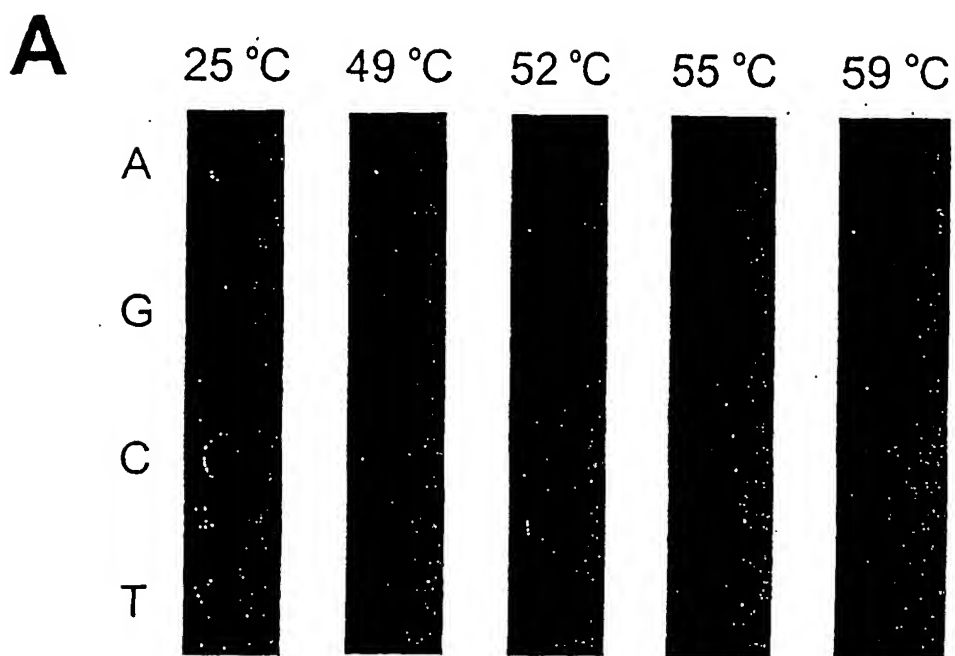


Figure 62

G. Lu, T. A. Taton and C. A. Mirkin



G. Lu, T. A. Taton and C. A. Mirkin

Figure 63

FIG. 55

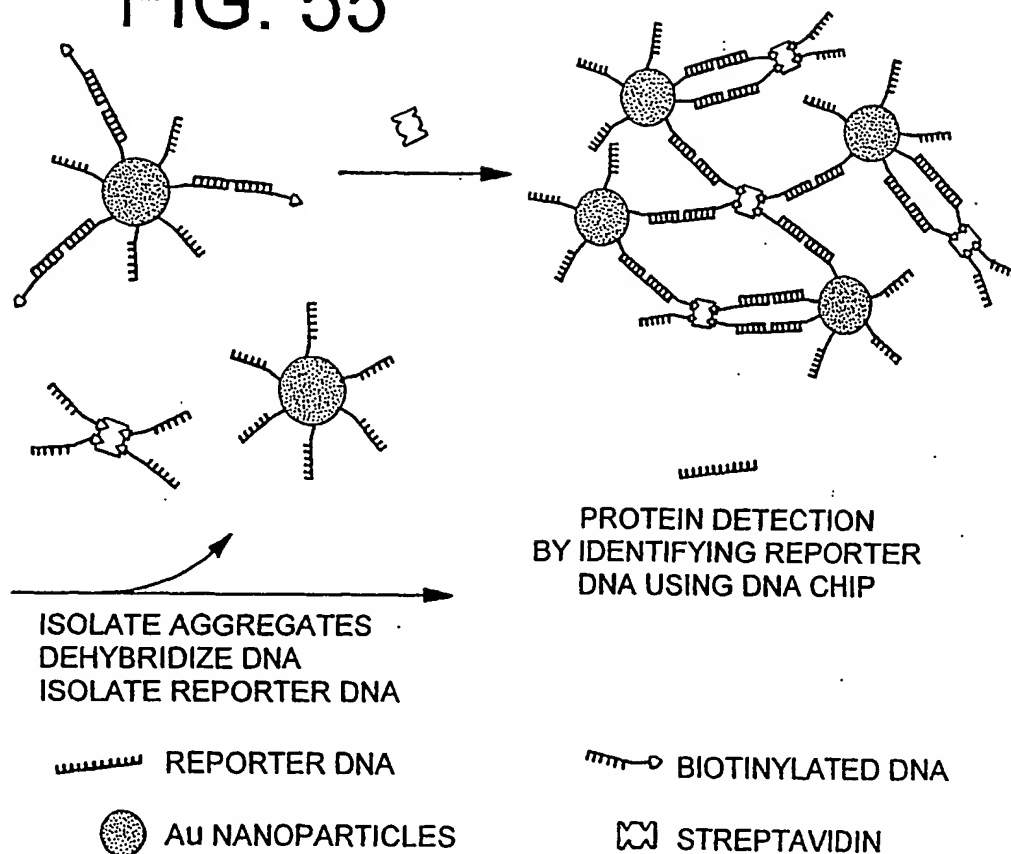


FIG. 56

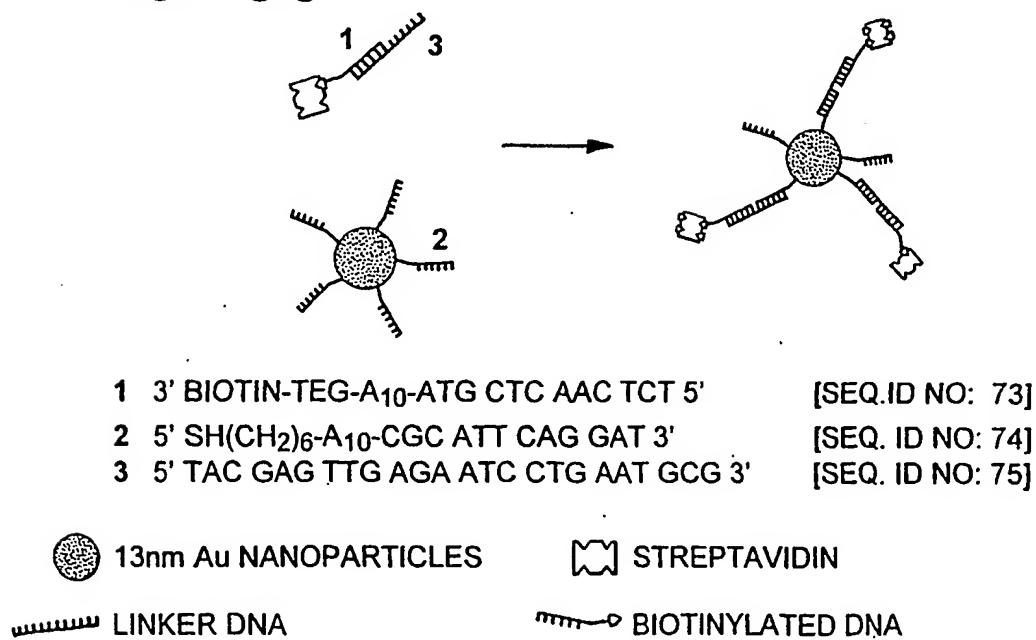


FIG. 57A

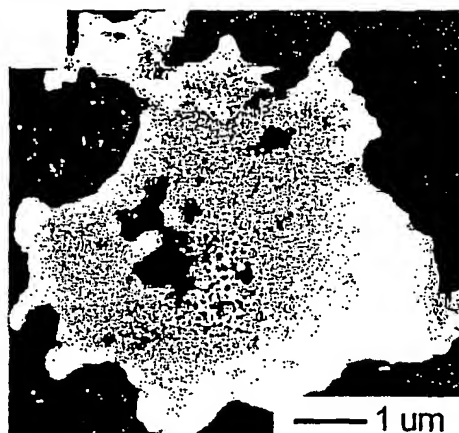


FIG. 57B

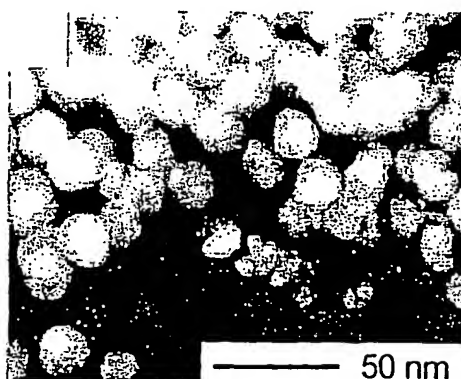


Figure 64

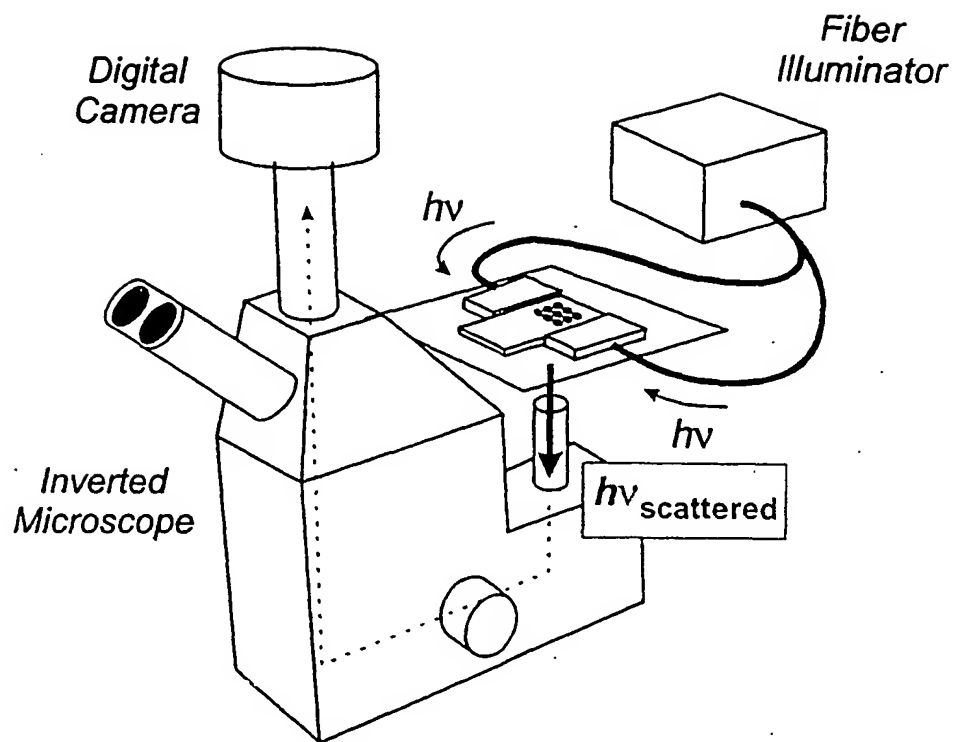
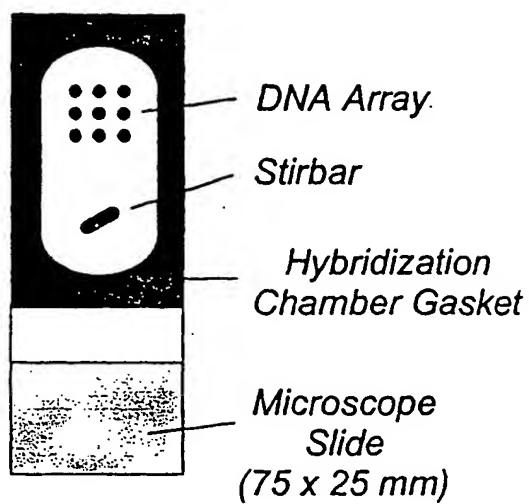
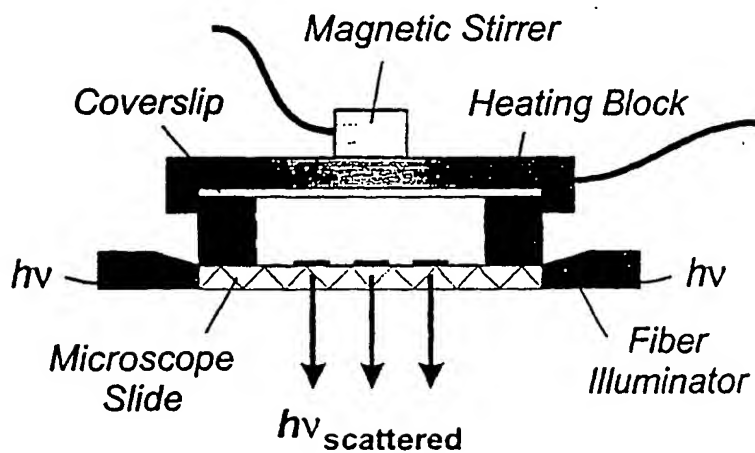


Figure 65

S9

**A****B**

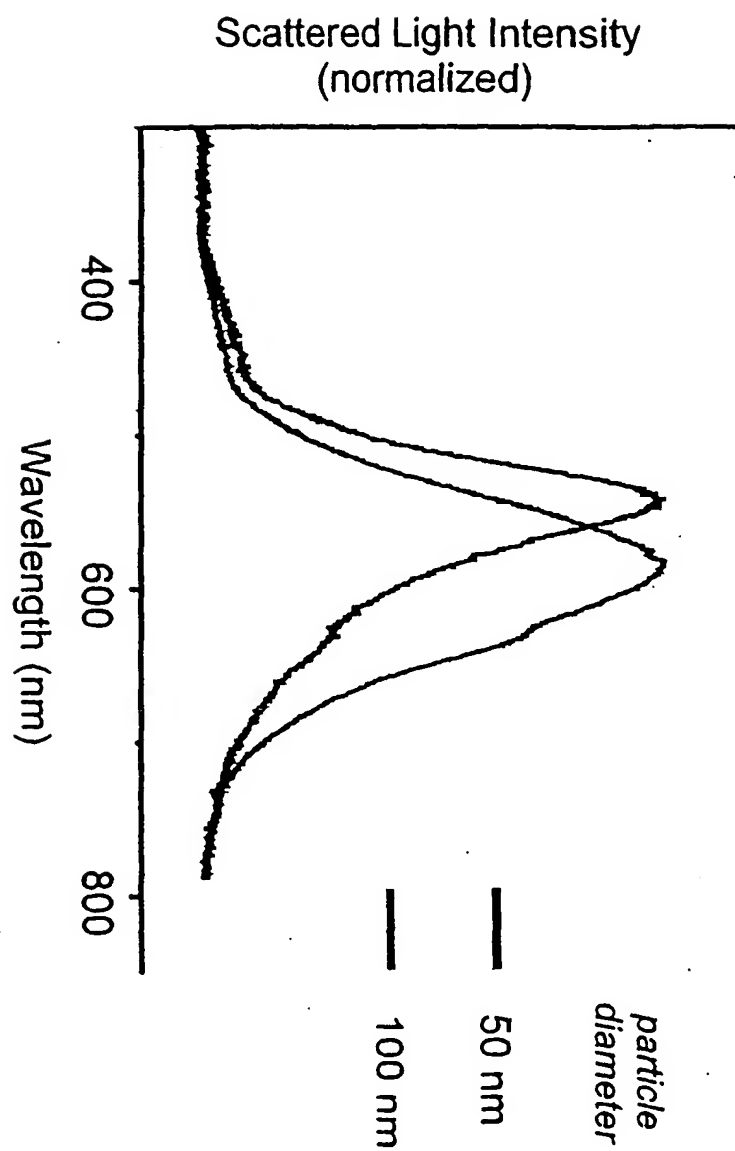
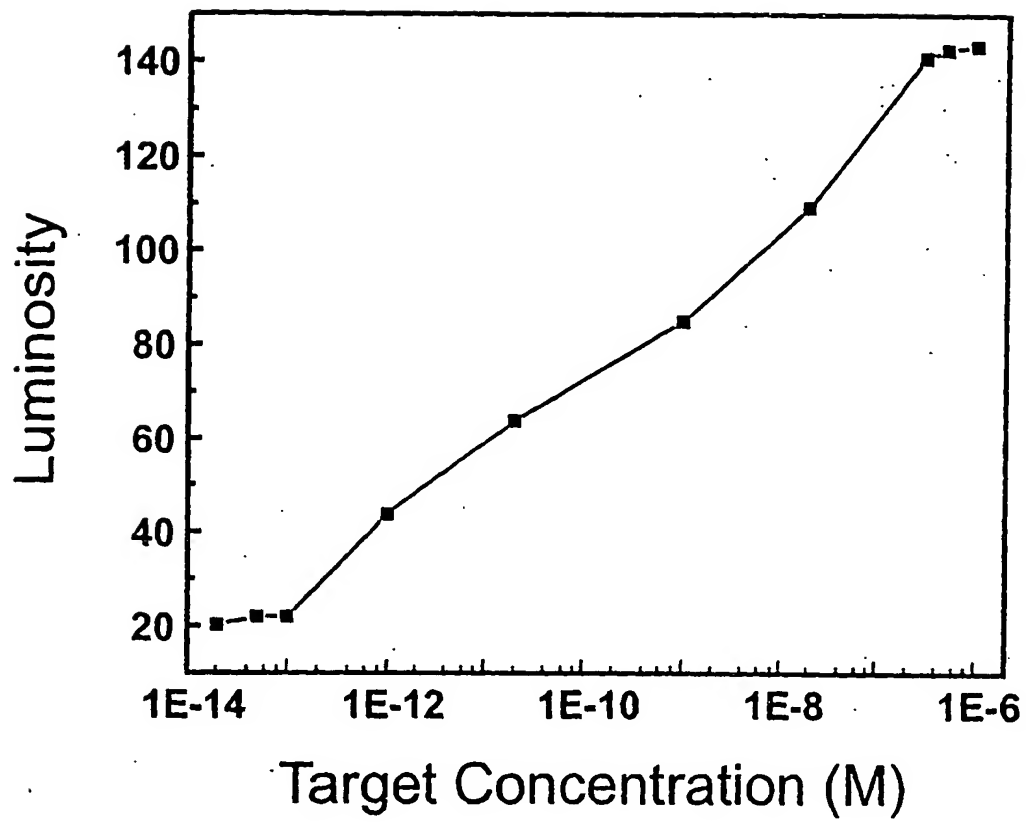


Figure 6b

*Figure 67*



## SEQUENCE LISTING

<110> Nanosphere, Inc.  
Mirkin, Chad A.  
Letsinger, Robert L.  
Mucic, Robert C.  
Storhoff, James J.  
Elghanian, Robert  
Taton, Thomas A.  
Garimella, Viswanadham  
Li, Zhi  
Park, So-Jung

<120> NANOPARTICLES HAVING OLIGONUCLEOTIDES ATTACHED THERETO  
AND USES THEREFOR

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<150> 60/254,392  
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16

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16

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26

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15

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15

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15

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28

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28

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15

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30

<210> 16  
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<400> 16  
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24

<210> 17  
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<400> 19  
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24

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<211> 48  
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48

<210> 33

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tctcaactcg ta

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<400> 34

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24

<210> 35

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12

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<211> 15

<212> DNA

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## synthetic sequence

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<211> 15  
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<220>  
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<400> 38  
atccttatca atatt 15

<210> 39  
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<212> DNA  
<213> Artificial Sequence

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<400> 39  
tgagcctcct taactactga ctcacccgcc 30

<210> 40  
<211> 25  
<212> DNA  
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<210> 41  
<211> 27  
<212> DNA  
<213> Artificial Sequence

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<210> 46  
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## synthetic sequence

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<211> 12  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:random  
synthetic sequence

<400> 50  
cgcattcagg at 12

<210> 51  
<211> 32  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:random  
synthetic sequence

<400> 51  
aaaaaaaaaa aaaaaaaaaa cgcattcagg at 32

<210> 52  
<211> 32  
<212> DNA  
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## synthetic sequence

<400> 52  
cgcatcagg atwwwwwww wwwwwwwww ww 32

<210> 53  
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synthetic sequence

<400> 53  
atcctgaatg cg 12

<210> 54  
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<400> 54  
atcctgaatg cg 12

<210> 55  
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<400> 55  
aaaaaaaaa aaaaaaaaaa 20

<210> 56  
<211> 27  
<212> DNA  
<213> Anthrax

<400> 56  
ggattattgt taattattga taaggat 27

<210> 57  
<211> 12  
<212> DNA  
<213> Anthrax

<400> 57  
taacaatnat cc 12

<210> 58  
<211> 15

<212> DNA  
<213> Anthrax

<400> 58  
atccttatca atatt

15

<210> 59  
<211> 12  
<212> DNA  
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<220>  
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<400> 59  
tctcaactcg ta

12

<210> 60  
<211> 24  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:random  
synthetic sequence

<400> 60  
tacgagttga gaatcctgaa tgcg

24

<210> 61  
<211> 48  
<212> DNA  
<213> Artificial Sequence

<220>  
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synthetic sequence

<400> 61  
gcgtaagtcc taacgtacta acggagcaga attgccagag ttgagcat

48

<210> 62  
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<220>  
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synthetic sequence

<400> 62  
gcgtaagtcc tacaacaggc atttcgcagg ttatatacgt actaacggag cagaattgcc 60  
agagttgagc at 72

<210> 63  
<211> 24  
<212> DNA  
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<220>  
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synthetic sequence

<400> 63  
tgcattgattg cctcgtctta acgg 24

<210> 64  
<211> 48  
<212> DNA  
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<220>  
<223> Description of Artificial Sequence:random  
synthetic sequence

<400> 64  
gttgctcgta aagcgtccaa tatatgcatg attgcctcgt cttaacgg 48

<210> 65  
<211> 16  
<212> DNA  
<213> Artificial Sequence

<220>  
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synthetic sequence

<400> 65  
tatcgttcca tcagct 16

<210> 66  
<211> 15  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:random  
synthetic sequence

<400> 66  
ttgatcttcc gttct 15

<210> 67  
<211> 34  
<212> DNA  
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<220>  
<223> Description of Artificial Sequence:random  
synthetic sequence

<400> 67  
agaacggaaa gatcaacgag ctgatggaac gata 34

<210> 68  
<211> 30  
<212> DNA  
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<220>  
<223> Description of Artificial Sequence:random  
synthetic sequence

<400> 68  
aaaaaaaaaa gacagacctca 30

<210> 69  
<211> 31  
<212> DNA  
<213> Artificial Sequence

<220>  
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synthetic sequence

<400> 69  
aaaaaaaaaa cctatgtgtc g 31

<210> 70  
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<220>  
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synthetic sequence

<400> 70  
aaaaaaaaaa 20

<210> 71  
<211> 21  
<212> DNA  
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<220>  
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synthetic sequence

<400> 71  
cgacacatag gtgaggtctg c 21

<210> 72  
<211> 35  
<212> DNA  
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<220>  
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synthetic sequence

<400> 72  
aaaaaaaaaa atccttatca atatt 35

<210> 73  
<211> 22  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:random  
synthetic sequence

<400> 73  
tctcaactcg taaaaaaaaa aa 22

<210> 74

<211> 22  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:random  
synthetic sequence

<400> 74  
aaaaaaaaaa cgcattcagg at 22

<210> 75  
<211> 22  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:random  
synthetic sequence

<400> 75  
aaaaaaaaaa cgcattcagg at 22

<210> 76  
<211> 24  
<212> DNA  
<213> Artificial Sequence

<220>  
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synthetic sequence

<400> 76  
tacgagtga gaatcctgat tgcg 24

<210> 77  
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<212> DNA  
<213> Artificial Sequence

<220>  
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synthetic sequence

<400> 7  
aaaaaaaaaa atccttatca atatt 35

<210> 78  
<211> 35  
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<220>  
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synthetic sequence

<400> 78  
aaaaaaaaaa taacaataat ccctc 35

<210> 79  
<211> 35  
<212> DNA  
<213> Artificial Sequence

<220>



<223> Description of Artificial Sequence:random  
synthetic sequence

<400> 79

taatatccctt cttataaaaa aaaaaaaaaa aaaaaa

35

<210> 80

<211> 35

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:random  
synthetic sequence

<400> 80

ctacaatata accctaataaa aaaaaaaaaa aaaaaa

35

<210> 81

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:random  
synthetic sequence

<400> 81

ataagaagga tattaaatat tgataaggat

30

<210> 82

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:random  
synthetic sequence

<400> 82

aggggttatat tgtaggaggg attattgtta

30

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